Innovations with lasers could lead regenerative dentistry

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_With the upcoming year, 2015, being designated as the year of light, the acknowledgment for the key role of light in multitude areas of our very existence and more specifically, in areas of human health are being widely promulgated.[1] Many references to the beneficial effects of light and specifically sunlight are replete in the literature across ancient civilisations.

Notably, the ability of concentrated light radiation in the management of lupus vulgaris by Niels Ryberg Finsen received the Nobel Prize in Medicine and Physiology in 1903.[2] The all-pervasive nature of opto-photoelectronics in our current society is readily evident such as the simplest supermarket laser scanners and optical communications to precision medical lasers and more recent laser weapon systems. This is also perhaps best highlighted by this year's Nobel Prize in Physics to the inventors of the blue light emitting diodes (LEDs), a simple invention with profound impact on our current society.[3]

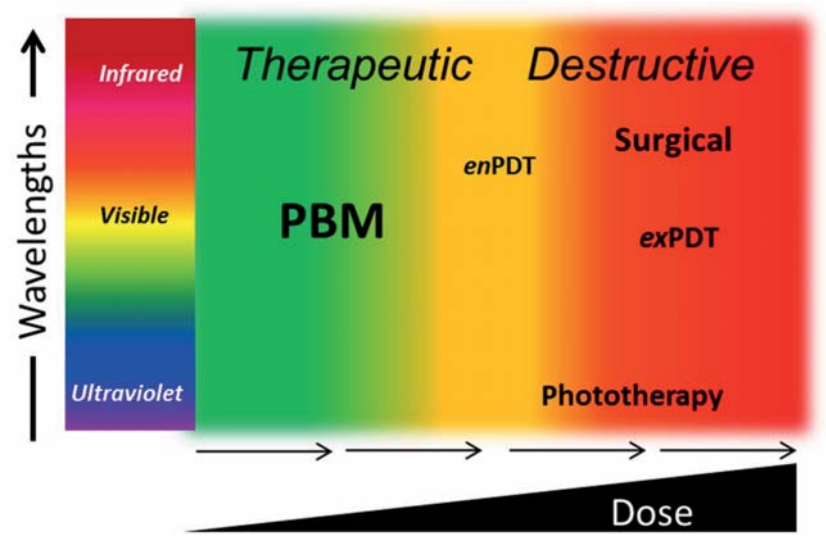

Fig. 1_The use of various wavelengths at different doses can be used for various clinical applications. The following acronyms are used in this figure PBM—Photobiomodulation; enPDT—Photodynamic therapy with endogenous chromophores and exPDT—Photodynamic therapy with exogenous chromophores (dyes).

_Clinical laser application

Dentistry has historically been a leading clinical specialty in adoption of new technologies. Light has been a central part of clinical dentistry from evolutions of operating lights and fibre optic illuminations to light cured restorations and more recently, optical imaging. Although lasers were commercially available since 1960's, the first dental laser for hard tissue applications was approved by the US FDA in 1997. Adoption for high power soft tissue applications has always been popular in many medical fields such as surgery, oncology, dermatology and ophthalmology.

First discoveries

Following the invention of this exciting new tool, early biological concerns focused around the safety of this new device with natural comparisons being drawn to ionizing forms of electromagnetic radiation. Among the early pioneering studies, Andre Mester reported a peculiar phenomenon—high doses destroyed tissue in a precise and predictable manner but very low doses produced a startling improvement in wound healing and promoted hair growth.[4, 5] This was a surprising discovery on many accounts.

While high energy electromagnetic radiation, such as Gamma, X-rays and Ultraviolet, were able

to achieve significant linear energy transfer generating biological damage (nucleic acid strand breaks), the effects of visible (and later infra-red) lasers did not appear to fall within these routine biological responses (Fig. 1). With much excitement, these initial observations spurred many investigations for the use of low powered lasers and other light devices (including filter-based broad light sources and LEDs) in many clinical and lab research studies.

Barriers in application

Unfortunately, a combination of the complexity of the early technology and a lack of understanding of its biological mechanisms has resulted in significant discrepancies in their reported therapeutic benefits. Hence, the lack of robust clinical efficacy has largely relegated the field to being side-lined as a pseudo-scientific and alternative medicine field. Current problems in the field from its basic terminology that prevents accurate indexing of the literature, to appropriate disease or biological response-specific clinical dose recommendations appear to be major barriers. Nonetheless, development of low power applications has also shown significant progress specifically in the areas of traumatic brain injury, post-traumatic stress disorders, reversal of methanol toxicity and wound healing.[6-15] In more recent years, mechanistic insights into light-biological tissue interactions have contributed to our better understanding for the therapeutic applications of laser therapy.[16-18]

Defining photobiomodulation

Our operational definition for Photobiomodulation (PBM) is a form of phototherapy that utilises non-ionizing sources (including broad light, LEDs and Lasers) in the visible and infrared spectrum that result in therapeutic benefits such as alleviation of pain or inflammation, immunomodulation and promotion of wound healing and tissue regeneration. PBM is a non-thermal process involving photophysical and photochemical events at various length scales resulting in beneficial photobiological responses. Its clinical applications could be appended as PBM therapy.

_Study 1: Activating TGF-β1

Based on prior reports, we began studies in 1999 to establish the parameters of the near infrared laser to effectively promote oral wound healing at low doses (3 J/cm^2, 10 mW/cm^2, 5 minutes). We performed a, thorough literature search to evaluate possible biological pathways involved in promoting wound healing. There appeared to be distinct correlations with reported use of exogenous TGF-β1 and laser treatments in wound healing.

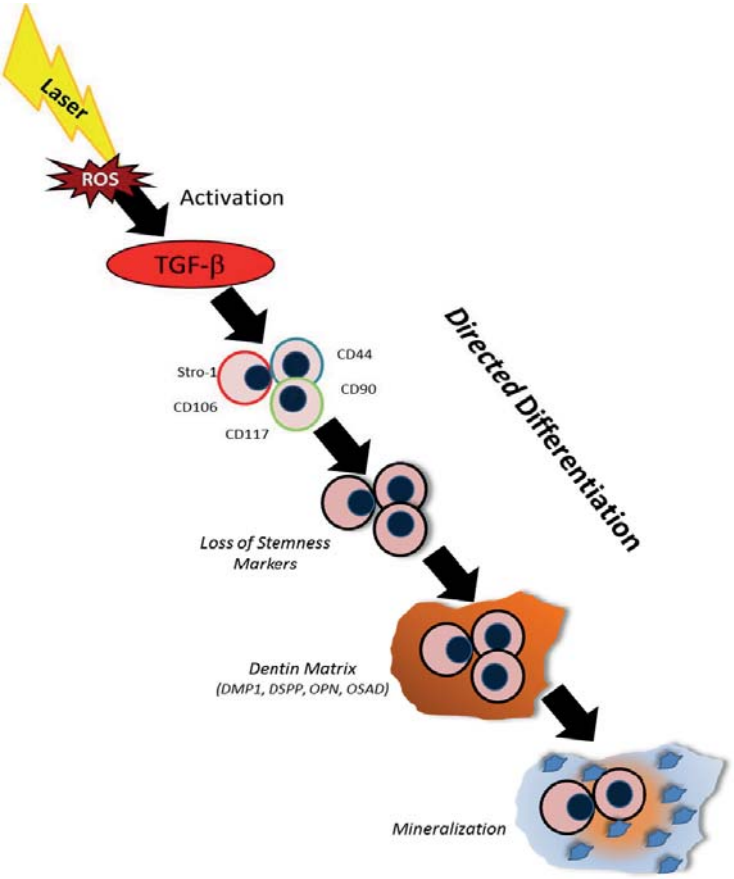

Fig. 2_Therapeutic outline utilizing laser-generated ROS activated TGF-β1 to direct differentiation of dental stem cells and pre-odontoblasts to induce dentin matrix and subsequent mineralization.

Based on these observations, we assessed the laser-treated healing response of oral tissues for TGF-β1 expression and noted increased expression immediately post treatment and at 14 days.[19] The increase at 14 days correlated well with an increased in monocyte-macrophage influx, well-known cellular sources of TGF-β1. We next looked into the increased early expression of active TGF-β1 in these wounds.TGF-β1 is secreted as a latent growth factor complex when associated with a Latency Associated Peptide (LAP). The activation process involves dissociation of LAP from active TGF-β1 dimer that is well-documented with a wide range of physio-chemical modalities such as proteases, extreme pH, heat, ionizing radiation and integrin binding among others. The early wound has abundant latent TGF-β from degranulating platelets present in the early wounds.

We observed low power laser treatments were capable of activating the latent TGF-β1 complex. To further pursue this observation mechanistically, we noted that near infra-red laser was capable of generating reactive oxygen species (ROS). This highly reactive, transient chemical intermediate was sensed by a key methionine residue on the latent TGF-β1 complex that resulted in a change in its conformation, resulting in its activation.[20]

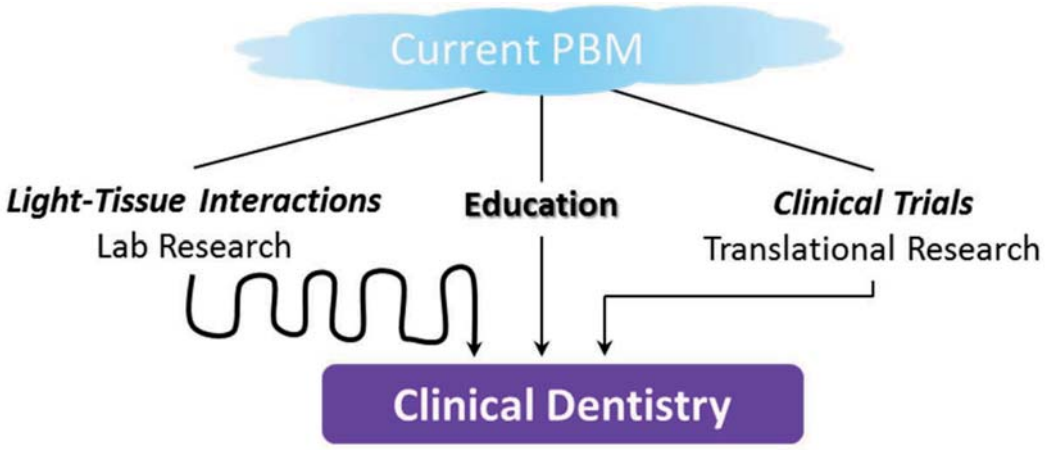

Fig. 3_Potential routes to move the field of PBM towards mainstream clinical dentistry. The wavy path from lab research to clinics is meant to reflect the multistep, tortuous basic science explorations in a wide range of topics that need to come together to aid in clinical translation.

_Study 2: Dentin regeneration

Having noted the effects of low power lasers on promoting oral mucosal wound healing in the prior study, we extended our clinical applications to dentin regeneration where TGF-β1 has been shown to play a pivotal role in dentin physiology.[21-25] We noted the ability of low power lasers to promote dentin regeneration using human dental stem cells. To validate these observations, rodent pre-odontoblasts (MDPC-23) cells grown in a polymeric scaffold, simulating a 3-D niche were treated with low power lasers.

Laser treatments were able to induce dentin differentiation as evident by increased dentin-specific matrix deposition and mineralisation. To confirm the role of TGF-β *in vivo*, transgenic mice with lack of TGF-β receptor in all cells capable of inducing dentin (utilising a Dentin Sialophosphoprotein specific transgene) were generated. Experiments in these mice did not demonstrate any significant dentin induction following laser treatment validating the critical role of TGF-β activation in mediating its effects.

Previous studies have shown the therapeutic benefits of supplementing exogenous (recombinant) TGF-β for reparative dentin, this study suggests the use of low power lasers can activate endogenous latent TGF-β1 present naturally in the pulp-dentin complex to drive differentiation of resident dental stem cells (Fig. 2). Thus, this therapy can utilise the inherent repair-regenerative responses naturally present in native tissues.

_Clinical Applications of Laser-Dentin induction

These observations have potent clinical implications where dentin would need to be therapeutically generated. The two directly relevant clinical scenarios are for pulp capping following deep carious lesions and for dentin desensitisation. In the former case, removal of decayed or damaged tooth structure approximating the pulp (close to or clear exposure) that require the use of pulp capping agents (such as Calcium hydroxide) could be potentially replaced with low power laser treatments.

In the second scenario, the use of low power laser treatments on exposed dentinal tubules could potentially generate an intrinsic dentin barrier that would relieve tooth sensitivity. This would be more effective than our current approach to extrinsically occlude exposed tubules modes.

The two major limitations of the current study were that we noted calcifications interspersed throughout the pulp chamber, spatially distinct from the laser-biological tissue interface. We believe this is perhaps a combination of the inherent near-infrared laser wavelength that readily permeates throughout biological tissue as well as the soluble nature of the activated molecules. This could be potentially addressed by better optical focusing techniques and use of specific reagents that absorb the radiant energy and spatially restrict the biological interphase.

A second limitation in this study was the observation that laser-generated dentin was a tertiary or reparative form that lacks pristine tubular structure. It appears that additional cues both biophysical (architecture) and biochemical (soluble, organizational), are likely necessary to promote morphodifferentiation of the newly induced dentin.

In attempts to further explore these molecular mechanisms, we have more recently extended developed a polymeric scaffold system with precise morphogen fields.[26] Using this model, we were able to extend our observations with dental stem cells and laser-activated TGF-β1 mediated dentin dif-

ferentiation to mesenchymal stem cells suggesting this approach could have significant potential with other stem cell types as well.

_Conclusion

Both ROS and TGF-β are central biological mediators in a wide range of biological responses.[27-29] The ability to selectively activate them in a spatio-temporally defined manner in vivo using low power lasers provides a significant clinical tool for various therapeutic interventions.

Questions on precise wavelengths, clinical protocol (delivery and dose ranges) and context of the pathophysiological response are all critical issues that need to be explored rigorously to enable further effective clinical translation of this therapy.[30] Further, the ability to effectively move this therapy into mainstream clinical dentistry will require more basic research, development of robust clinical standards and education at various levels (basic dental training and continued education) (Fig. 3).

In the current era of personalised medicine and strategies to utilise sophisticated technologies and pharmaceuticals to individualise health care, the significant promise of lasers in clinical dentistry may indeed be the leading, pivotal technology that ushers in the new era of regenerative dentistry.

Acknowledgement

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Editorial note: A list of references is available from the publisher.

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