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Implication of Heat Stress on Mesenchymal and Dental Stem Cells

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Abstract Iatrogenic temperature increases during dental procedures are able to damage dental tissues including dental stem cells (DSCs) in dental pulp, alveolar bone, and periodontal ligament. While a lot of research demonstrated the heat damage on dental tissues, there is ambiguity on the how heat stress impact the cellular response especially the regenerative potential of DSCs. In comparison, more research exists on the interplay of heat stress with mesenchymal stem cells (MSCs) that is informative to understand the underlying mechanisms. This review summarizes the clinical procedures that induce hyperthermia of dental tissues, and recent studies on the cellular effects of heat stress on MSCs and DSCs.

Keywords Heat stress, Mesenchymal Stem Cell, Dental Stem Cell

1. Introduction

Dental iatrogenic trauma can be defined as any trauma that has been induced by the dentist's activity, manner, or therapy [1]. Dental procedures that generate heat during the treatment include use of high and low torque handpieces, photopolymerization of composite restorative materials, fabrication of acrylic resin provisional crowns, thermoplasticized root canal obturation, ultrasonic removal of posts and fibers, and ultrasonic scaling [2,3]. The pulp and tooth-supporting tissues are not immune from the risks of temperature increase. For example, restorative and periodontics procedures can affect periodontal ligament and alveolar bone through an increase in root surface temperature [2]. As multiple dental procedures have the capability of surpassing critical temperature stress levels in dental tissues, the implication of the heat stress is pertinent in order to reduce and potentially to repair thermally damaged tissue.

Dental stem cells (DSCs) must retain their viability and stem cell properties induced by the thermal insult. While data exists on the heat induced damage on dental tissues, there is a lack of information on the cellular and molecular mechanism underlying heat stress effects in DSCs. Yet, due to coinciding properties of mesenchymal stem cells (MSCs) and DSCs, research conducted on the heat stress effects in MSCs may shed light on some predicted responses in DSCs.

Heat stress, also referred as heat shock in many experiments and procedures, is a major factor inducing oxidative disturbance in cells. Heat stress provokes the overproduction of reactive oxygen species (ROS) in cells [4], creates phenotypic changes in MSCs and gravely curtails their capacity to proliferate and differentiate [5].

On the other hand, heat stress induced elevation of heat shock proteins, which mediate protective effects in MSCs. Several previous studies have focused on the impact of sub lethal exposure to stressful environments, such as hypoxia, heat, and nutrient depletion, which showed that preconditioning procedures protect MSCs from the altered environments [6–8].

In this review, we summarize the origins of the dental thermal irritation and discuss the heat stress effects on MSCs and DSCs. The information will be helpful on the prediction of a potential ‘safe heat shock’ to protect stem cells and promote tissue repair.

2. Dental Procedures Inducing Heat Stress

2.1. Restorative Tooth Preparation

Restorative procedures can increase intrapulpal temperature. During restorative tooth preparation with high and low torque handpieces, frictional heat is created between the bur and tooth. The level of heat stress in the dental pulp is influenced by the residual dentin thickness, type of handpiece used, the handpiece rotation speed, the type and shape of the bur, the amount of pressure exerted on the handpiece, the length of time in contact with the dentin, and the usage of water cooling [2,9]. Studies also showed that with less residual dentin thickness the intrapulpal temperature increase was greater [10,11]. With a residual

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dentin thickness of 0.5 mm, the maximum temperature rises in the pulp was 0.8℃ and 1.8℃ in different studies [10,11]. Further, regardless of the pressure exerted, sufficient water-cooling is essential to keep the intrapulpal temperature below the reference critical temperature. Insufficient water-cooling led to 19.7℃ rise for high-speed handpieces and 5.5℃ rise for low-speed handpieces [12]. While warm water spay above 35℃ resulted in intrapulpal temperature at 44.4℃ [13].

2.2. Light Curing Composite Resin

Another restorative dental procedure that generates heat is light curing of composite resins. In light curing, heat is both generated from the light curing unit as well as the exothermic reaction of the composite resin [14]. In a real-time temperature monitoring study, a 9.6° to 10.3℃ temperature rise was detected during nano- and micro-hybrid commercial composite light curing using a blue-violet light emitting diode (LED) curing light. The study also showed that the temperature increase can be variable depending on the type of resin utilized, as flowable commercial composites recorded a 12.5℃ temperature rise [15]. Another study compared LED low mode, LED high mode with quartz-tungsten halogen (QTH) among unprepared tooth, 1.8mm depth Class V cavity, cavity with filled resin composite, cavity with flowable composite. LED high mode led to intrapulpal temperature rise surpassed the 5.5℃ critical temperature in all groups, with the highest intrapulpal temperature (45.3℃) observed in cavity with flowable composite group [16].

2.3. Acrylic Resin Provisional Crowns

The creation of provisional crowns with acrylic resin produces an exothermic reaction during the setting process. A study measuring the temperature change of the pulp chamber recorded 7.2℃ to 12.3℃ temperature rises following acrylic resin usage [17]. One study analyzed temperature rises in pulp cavity using autocuring provisional resins prepared at room temperature, 4℃, and -12℃ and a clear polyethylene matrix. While the 4℃ and -12℃ putty matrix groups induced no temperature rise, the room temperature putty matrix led to a 3.4℃ to 5.5℃ rise and the clear polyethylene matrix led to a 4.0℃ to 8.2℃ rise [18].

2.4. Endodontic Procedures

Endodontic procedures also cause temperature increases. Warm vertical gutta percha obturation using battery-generated heat and flame-heated carriers have displayed over 10℃ temperature rise at root surfaces [19]. Both red-light (630nm) and blue-light (380-500nm) chromophores generate ROS to induce antimicrobial death and are useful for endodontic disinfection; however, red-light chromophores pose a risk of heat stress to oral tissues while blue-light chromophores do not [20]. Ultrasonic removal of posts also produces heat via friction between an ultrasonic tip and titanium post and via acoustic energy absorption of the ultrasound [21]. An *in vitro* study showed that water coolant and evacuation both reduced the temperature at the post and root surfaces during ultrasonic post removal, with a stronger reduction using water coolant. In the absence of the coolant but with evacuation, temperature rise at the post surface was around 20 - 30℃ and at the root surface around 10 - 20℃ after 120 second use of the piezoelectrical ultrasound transducer. In comparison, magnetostrictive ultrasound transducer led to much smaller temperature rise [22].

2.5. Ultrasonic Scaling

Ultrasonic scalers used in dentistry to remove deposits from tooth surfaces can produce heat via frictional heating through scaler and tooth contact as well as via acoustic energy absorption of ultrasound into the tooth [3]. Similarly, sufficient coolant in quantity of 30 mL/min limited the temperature rise to 4℃ at the dentin surface; however, without the coolant the temperatures could rise by 35℃ [23]. While frictional heat of ultrasonic scaling was show to lead temperature rises of only 2℃ in pulpal chamber due to acoustic absorption [24].

As the duration of the temperature elevation and total amount of heat generated during the period can be difficult to measure *in vivo*, most of the reported data were acquired on extracted teeth *in vitro*, which might partially account for the discrepancy of critical temperature in different studies. In addition, the methods and design of the study and variation of extracted teeth all contribute to the determination of the critical temperature. Further investigation is warranted to better understand the association of dental procedure induced heat stress and potential damage on the dental, periodontal and surrounding oral tissues.

3. Effects of Heat Stress on Mesenchymal and Dental Stem Cells

3.1. Mesenchymal Stem Cells

Stem cells are known for their properties of self-renewal, proliferation and multilineage differentiation [25]. MSCs are identified in many tissues including the skin, bone marrow, peripheral blood, brain, liver, and dental tissues [26]. As MSCs are long lived in multicellular organisms, they are at increased risk of accumulating cellular and genetic damage from intrinsic or extrinsic factors including the heat stress.

Heat shock proteins (HSPs) are the most well studied cellular responders to heat stress. Expression of HSPs is upregulated when cells are exposed to heat as well as other stress inducing factors such as anoxia, viruses, noxious chemicals, and surgery. As molecular chaperons, HSPs play important roles in regulating refold and repair denatured proteins, degrade injured proteins, prevent misfolded protein aggregation, and guarantee proper folding of newly created protein. Heat stress could disrupt the assembly and folding of proteins, resulting in an increased amount of unfolded

proteins that further enhance transcription and translation of HSPs [27].

Hsp70 gene expression elevated as soon as 2 hours after 45℃ heat stress for 30 minutes in MSCs derived from human shed endometrium. Interestingly, HSP70 protein level returned to normal more rapidly in quiescent cells than in duplicating cells, which might account for higher resistance of quiescent MSCs to heat stress. In addition, viability of MSCs was not affected bv the heat stress, but premature senescence was induced by heat stress as determined by increased beta-galactosidase activity and increased p21 protein [25]. Along the same line, MSCs overexpressed Hsp70 can enhance the capacity and efficacy of MSCs in the treatment of phosgene-induced acute lung injury (ALI) [28]. Moreover, recombinant human Hsp70 at concentrations of 2 ug/ml and higher significantly enhances growth of MSCs from aged mouse in culture. An identical result was observed by application of a mild heat shock at 42℃ for 5 minutes to the cells [29].

The strength and duration of the heat stress has bigger impact on the cellular response from MSCs compared to the intrinsic factors such as cell cycle. High temperature can be detrimental to cellular activity even for short duration of exposure. Human MSCs were exposed to 38℃, 48℃, and 58℃ with 45, 80, and 150-second durations. 58℃ exposure for as short as 45 seconds led to significant decrease in metabolic activity and irreversible cell damage. On the other hand, 48℃/80-seconds and 48℃/150-seconds significantly reduced cell viability 3 days and 7 days after the heat shock, but the percentage of the viable cells gradually increased in culture, indicating the surviving cells successfully proliferated [30].

This raises the question of whether the MSCs could return to functional normalcy after sublethal heat stress. An *in vivo* study showed that 45℃ heat shock for 30 minutes induced random chromosome breakages and aneuploidy in endometrial MSCs. However, oncogenic signaling pathway was downregulated while tumor-suppressing pathway was upregulated [31].

Study mimicking heat stress during orthopaedic cutting during surgery used osteoblast-like MC3T3-E1 cells that were exposed at 37℃, 45℃, 47℃ or 60℃ for 30 seconds and 1 minute. Calcium deposition and expression of osteogenic differentiation marker significantly increased after heat shock at 45℃ without a decrease of cell viability. Although heat shock at 60℃ significantly reduced cell viability, the surviving cells demonstrated stronger osteodifferentiation potential than cells exposed to milder heat shock [32].

Likewise, heat shock between 20 minutes and a few hours at 42 - 43℃, has been reported to be advantageous in MSCs transplantation [33–37]. It is also reported that heat stress induced elevation of HSP70 expression and reduced cytotoxicity of neural progenitor cells [38].

In addition, the interval of heat stress may also play a role in regulating cellular responses. Constant heat stress at 40.5℃ for 24, 48, or 72 hours and pulse heat shock at 39℃ or 42℃ for 1 hour induced premature senescence of bovine MSCs, without decreasing viability. Constant heat stress depreciate MSC immunomodulatory function, while pulse heat shock positively correlated with high level of reactive oxygen species (ROS) [5], which could evoke stem cell depletion [39].

These reports indicate that mild heat stress could have beneficiary effects, although more evidence is needed to further specify the safe temperature range, duration, and mode of exposure, as well as the biological effects in different types of MSCs.

3.2. Dental Stem Cells

DSCs are a type of MSC extensively studied in regenerative medicine and tissue engineering fields as they can be easily isolated [40]. Eight MSC populations have been identified in dental tissues so far, including the postnatal human dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), alveolar bone marrow stem cells (ABMSCs), dental follicle stem cells (DFSCs), stem cells from dental apical papilla (SCAPs), gingiva-derived mesenchymal stem cells (GMSCs), stem cells from human exfoliated deciduous teeth (SHEDs), and tooth germ progenitor cells (TGPCs) [41].

Heat stress can induce mild injury in dental pulp as localized subodontoblastic hemostasis and hemorrhage, moderate injury as displacement of odontoblasts as well as considerable changes in pulp microcirculation, and severe injury as total destruction of the odontoblastic layer [10]. For the periodontium, mild thermal injury presents as protein denaturation and ankylosis while more severe injury results in necrosis of both the periodontal ligament and alveolar bone [42,43].

Previous studies have reported heat stress that outline critical temperatures for the dental tissue [2,44]. An study on Rhesus monkey reported that a 5.5℃ intrapulpal temperature increase caused 15% irreversible pulpal damage [44]. The maximum critical temperature rise that dental pulp can withstand was suggested to be 5.5℃ in many following studies [45]. An *in vivo* study on rats showed that heat induced pulp damage is perceptible starting at 5℃ [46]. While a clinical trial indicated that temperature elevation from 8.9° to 14.7℃ did not result in pulpal necrosis. However, in most studies the temperature change was measured in extracted teeth to mimic the heat stress in the patients [47]. Regarding the periodontal ligament and alveolar bone, it was reported that a rise of 10℃ on the exterior root surface resulted in ankylosis of the tooth and alveolar bone resorption [48].

Despite numerous studies on the histological presentation of heat induced damage to the dental pulp and supporting tooth structures, less research has focused on the impact of heat stress on the regenerative activity of DSCs.

Like MSCs, higher temperature at 42℃ induced cellular toxicity in DPSCs after 30 minutes exposure, and down regulated the P2Y1 pathway that is important in cell migration and inflammatory responses. Hence DPSC

migration was significantly inhibited 24 hours after the heat shock [49]. On the other hand, mild heat stress induced positive regenerative activities in DSCs. Proliferation and osteogenic differentiation increased in DFSCs cultured at 38 - 40℃ than DFSCs cultured at 37℃, while both activities were inhibited at 41℃ [50].

As for inflammatory responses, heat stress at 42℃ for 30 minutes induced the production of ROS and proinflammatory cytokines in DPSCs [51]. Heat stress at 42℃ for 30 minutes synergistically increased the expression of silent information regulator 1 (SIRT1), a stress response regulator, with lipopolysaccharide treatment in DPSCs [52]. In addition, synthesis of leukotriene B4 (LTB4), a proinflammatory arachidonic acid mediator, was significantly elevated in DPSCs following heat shock at 38°, 39°, 40°, 42°, and 45℃ for up to 30 seconds [53].

4. Conclusions

Multiple dental procedures have the potential of surpassing critical heat stress levels in dental tissues. Due to shared properties of MSCs and DSCs, research conducted on MSCs may provide clues to understand the cellular response to heat stress at different strength and duration. Mild heat stress is promising to promote regenerative responses in DSCs. Although different mechanism may present in different types of cells, or homogenous cells at different status. It would be necessary to further investigate the strength, duration as well as the delivery method of different dental procedure in relation to the DSCs in the future. The information may lead to "safe heat shock" that can be a promising therapeutic strategy to protect DSCs and promote tissue regeneration.

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Disclosure

The authors declare no conflict of interest.

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