



QUALITY IMPROVEMENT IN DENTAL AND MEDICAL KNOWLEDGE, RESEARCH, SKILLS AND ETHICS FACING GLOBAL CHALLENGES

Edited by

Armelia Sari Widyarman, Muhammad Ihsan Rizal,
Moehammad Orliando Roeslan & Carolina Damayanti Marpaung



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QUALITY IMPROVEMENT IN DENTAL AND MEDICAL KNOWLEDGE, RESEARCH, SKILLS AND ETHICS FACING GLOBAL CHALLENGES

The proceedings of FORIL XIII 2022 Scientific Forum Usakti conjunction with International Conference on Technology of Dental and Medical Sciences (ICTDMS) include selected full papers that have been peer-reviewed and satisfy the conference's criteria. All studies on health, ethics, and social issues in the field of dentistry and medicine have been presented at the conference alongside clinical and technical presentations. The twelve primary themes that make up its framework include the following: behavioral epidemiologic, and health services, conservative dentistry, dental materials, dento-maxillofacial radiology, medical sciences and technology, oral and maxillofacial surgery, oral biology, oral medicine and pathology, orthodontics, pediatrics dentistry, periodontology, and prosthodontics. This proceeding will be beneficial in keeping dental and medical professionals apprised of the most recent scientific developments.



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Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges

Edited by

Armelia Sari Widyarman, Muhammad Ihsan Rizal,
Moehammad Orliando Roeslan and Carolina
Damayanti Marpaung
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Preface

Faculty of Dentistry Universitas Trisakti (Usakti) presents FORIL XIII 2022 Scientific Forum Usakti conjunction with International Conference on Technology of Dental and Medical Sciences (ICTDMS) on December 8th–10th 2022. The theme of the conference is “Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges”.

The triennial conference has served as a meeting place for technical and clinical studies on health, ethical, and social issues in field medical and dentistry. It is organized around 12 major themes, including behavioral, epidemiologic, and health services, conservative dentistry, dental materials, dento-maxillofacial radiology, medical sciences and technology, oral and maxillofacial surgery, oral biology, oral medicine and pathology, orthodontics, pediatrics dentistry, periodontology, and prosthodontics.

The most recent findings in fundamental and clinical sciences related to medical and dental research will be presented in the conference that will be published as part of the conference proceeding. This proceeding will be useful for keeping dental and medical professionals up to date on the latest scientific developments.

Dr. Aryadi Subrata
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Various compounds that are used as oxidative stress inducers on fibroblast cell

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ABSTRACT: To elucidate the effect of oxidative stress on fibroblast cells, it is crucial to create an oxidative stress model in research *in vitro* as close to the natural pathologic condition. The objective of this study is to evaluate compounds used as oxidative stress inducers on fibroblast cells, the concentrations used, and the signaling pathways investigated. This research's systematic review method was based on the PRISMA guidelines and used the data basis on PubMed publications from 2020 to 2022. There were 431 records identified. Postscreening, there were 179 records left, and 81 passed as eligible, then after the final screening, there were 30 articles included in this research, and from each of the article, information about compound names, compound concentrations used, and pathways that were investigated were collected. From 30 records, hydrogen peroxide (H₂O₂) was the most used compound to induce oxidative stress, followed by type B ultraviolet (UVB), type A ultraviolet (UVA), and tumor necrosis factor-alpha (TNF- α). The most used concentration for H₂O₂ was at 100–200 μ M, meanwhile for UVB, intensity was at 10–1000 mJ/cm². The most used signaling pathway in the records was the nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), followed by mitogen-activated protein kinase (MAPK) and nuclear factor erythroid 2-related factor 2 (Nrf2). The compounds, concentrations, and pathways that were used/investigated on each of the records differ depending on the study's design based on the study's objective created by the respective researchers.

1 INTRODUCTION

Fibroblasts are the most common connective tissue cell type in animals, exhibiting elongated morphology and extensive cell processes with a spindle-like shape (Yin et al. 2022). These cells play important roles during tissue development, differentiation, and repair in many organs. In the proliferative phase of the wound healing process, for example, fibroblasts will proliferate in the wound area and synthesize collagen fibers and other protein matrixes, which will cover the wound (Tarin & Croft 1969). Because fibroblasts play many important roles, and it is easy to be cultured *in vitro*, these cells often are used in many studies to investigate disease pathogenesis (Tarin & Croft 1969; Tomasek et al. 2002).

Injuries can be caused by microbial infections, physical factors, chemicals, or hypersensitivity reactions, when they occur, the body will react to these stressors. This is simply what is known as the inflammatory state (Cohen & Hargreaves 2011). The inflammatory state increases cellular metabolic reactions. The waste products of the metabolic process are known as reactive oxygen species (ROS). The ROS is a combination of oxygen groups that are

partially reduced metabolites, like superoxide and hydrogen peroxide. The presence of ROS in cells can help fight pathogens by acting as a trigger for the activation of intracellular signaling, for example, in triggering phagocytosis (Patel et al. 2018). Oxidative stress is a condition in which there is an overproduction of ROS, surpassing the production of antioxidants. This condition can interfere with the function of cells and cause tissue damage (Janda et al. 2016).

There are still many oxidative stress effects that are still unclear or unknown. To elucidate the effect of oxidative stress on fibroblast cells, the researcher must be able to create an oxidative stress model *in vitro*, as close to the natural pathologic condition. Hence, this systemic review aims to evaluate compounds used as oxidative stress inducers on fibroblast cells, the concentrations used, and the signaling pathway investigated in some studies from PubMed within a certain timeframe. We believe this is the first scoping review to enlist the compounds used to induce oxidative stress on fibroblast cells.

2 METHODS

2.1 Eligibility criteria

Studies were considered to be eligible for inclusion if the method(s) or aim(s) of the studies included inducing ROS on fibroblast cells *in vitro*. The studies must mention at least one compound, protein, or energy with its dose, concentration, or intensity used as the technique to induce the ROS in fibroblast culture. An electronic search strategy with combined keywords and indexing vocabulary (MeSH terms) was conducted in the PubMed database on the Advance search interface. The following search terms were used: 'ROS' and 'fibroblast cell'. From 2020 to 2022 and only English-written articles are included.

2.2 Study selection and quality assessment

The search and selection process is shown in Figure 1. There were 431 records identified using the electronic search strategy. Review articles, nonfull text, and duplication were then

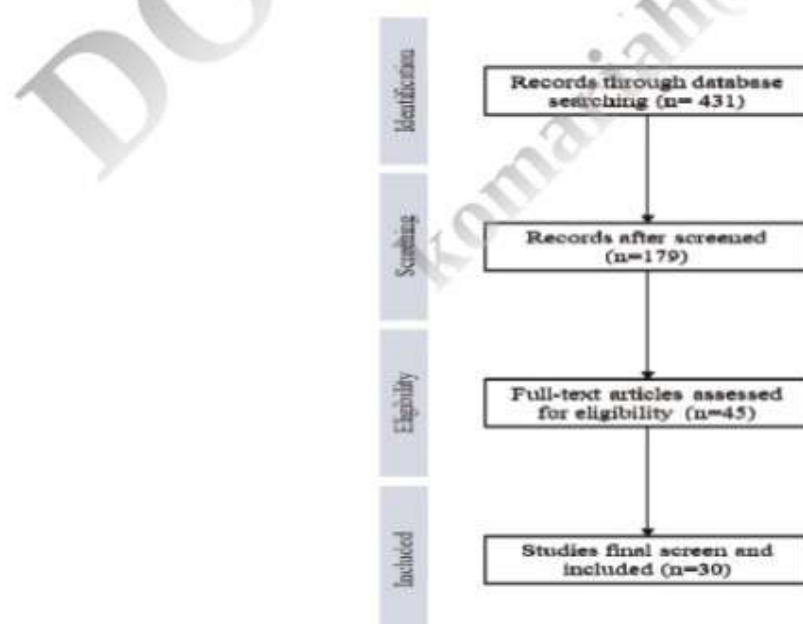


Figure 1. The flowchart of depicting the systematic selection and exclusion of articles related to the topic.

removed, making only 179 records remain. The off-topic articles were then discarded, and only the articles deemed appropriate were assigned for full-text evaluation (n=30, Supp. Table). The exclusion reasons of the articles are: studies not on human fibroblast cells, no exact concentrations or intensity mentioned in the methods, the studies were off-topic, no clear distinction on how to induce the ROS level, articles are review articles, editorials, comments, or abstract only.

2.3 Data extraction and analysis

The information regarding reference names, studies' objectives, information about compound names used to induce ROS, compound concentrations used, and pathways that were investigated was collected. From the database collected, methods of how to induce ROS and the signaling pathway investigated were summarized. The data collected were then discussed.

3 RESULTS

From 30 articles collected, 11 of them used H₂O₂ as the ROS inducer to create oxidative stress on fibroblast cells. The second most used method to induce ROS was UVB induction, followed by UVA induction and TNF- α treatment as the third and fourth most used methods to create oxidative stress (Table 1). Of 11 articles that used H₂O₂, 8 articles used a concentration of around 100-200 μ M, and 3 articles used a concentration of more than 200 μ M. Meanwhile, 7 articles used UVB induction, 6 articles used an intensity around 10-1000 mJ/cm², and 1 article used an intensity of more than 1000 mJ/cm² (Table 2). The most investigated signaling pathway was the NF κ B signaling pathway, with an n value of 11. The second most investigated pathway from 30 articles was MAPK and Nrf2 signaling pathway (Table 3).

Table 1. The list of methods used to induce oxidative stress.

No	Methods used for ROS induction	n
1	H ₂ O ₂	11
2	UVB	7
3	UVA	3
4	TNF- α	2
5	Particulate matter (PM)	2
6	COCl ₂	1
7	LPS	1
8	Bacteria lysate	1
9	Alendronate sodium salt trihydrate	1
10	NADPH	1
Total		30

Table 2. The concentration used by the two most used methods for ROS induction.

No	Compound name	Concentration	n
1	H ₂ O ₂	100-200 μ M	8
		>200 μ M	3
2	UVB	10-1000 mJ/cm ²	6
		>1000 mJ/cm ²	1

Table 3. The list of pathways that were investigated among the records.

No	Pathway	n
1	NfKb	11
2	Nrf2	7
3	MAPK	7
4	Akt	1
5	RANKL	1
6	TGF- β 1/SMAD	1
7	bax bcl-2 pathway	1
8	aMPK/Sirt1	1
	Total	30

4 DISCUSSION

Fibroblasts play an important role during tissue development, differentiation, and repair in many organs. Fibroblasts are capable of producing ROS during the proliferative phase, as are neutrophils and macrophages, but an excessive increase in ROS production can affect the proliferation process (Janda et al. 2016). This makes fibroblasts a very important target cell in healing therapy (Rodriguez et al. 2019).

Oxidative stress occurs when the cell's ability to eradicate the free radicals is less than the degree of free radicals formation itself (Pizzino et al. 2017). Left unchecked, this imbalance can create problems, such as triggering or progressing some diseases like diabetes, cancer, or/and metabolic disorder (Taniyama, Y., & Griendling 2003). However, recent studies also showed that with the right degree of ROS level and accurate oxidative stress exposure time, it can be beneficial for the treatment of some cancers (Dent et al. 2003; Ward 1988). These opposite findings proved that there are still many parts to be unveiled in the ROS-oxidative stress condition. Hence, it is important to be able to create an oxidative stress model *in vitro* as close as possible to the clinical condition.

The result of this study showed that most of the records collected used H₂O₂ to create oxidative stress. In human cells, mitochondria become the main producer of ROS, both during healthy conditions and pathologic conditions (Pizzino et al. 2017). The H₂O₂ is produced when mitochondria produce ATP, which will generate superoxide (O₂⁻) as the byproduct. The superoxide will be generated H₂O₂, which will (in normal circumstances) be hydrolyzed by antioxidants such as glutathione peroxide to become water (Choi & Kim 2019). The compound of H₂O₂ is considered to be different among common toxins, because H₂O₂ has high stability in inorganic environments and neutral pH, yet can eliminate any type of cells by creating very reactive hydroxy radicals (Mahaseth & Kuzminov 2017). This is why H₂O₂ is commonly used to induce oxidative stress.

The second and third most used method to induce ROS is exposure to UVB and UVA. Naturally, ultraviolet radiation by the sun made the fact that most humans on this planet have been exposed to it (De Jager et al. 2017). The UV radiation will affect the catalase enzyme, which will induce the expression of nitric oxide synthase (NOS), which is one of the free radicals that, if left unchecked, can induce oxidative stress (Brüne et al. 2003). The difference between UVA and UVB is in its wavelength. Because they proved to be able to create oxidative stress, both contributed to the development of skin aging and skin cancer (Amaro-Ortiz et al. 2014). Because fibroblasts are the most

common cells found in connective tissue, many studies investigating the progress of skin disease used UV as the inducer of oxidative stress (Dare et al. 2020; Lion et al. 2021; Zhang et al. 2020).

The following method used the recombinant protein of TNF- α , which is a proinflammatory cytokine that played a major role in the inflammation process (Jang et al. 2021). Inflammation is a nonspecific immune response as a reaction to all types of injury caused by microbial infection, physical factors (trauma, radiation, and temperature), chemicals (irritant and corrosive chemicals), as well as tissue necrosis and hypersensitivity reactions (Tania 2018). Cross talk is common to occur in inflammation conditions, some signaling pathways can be activated by TNF- α , such as the Nrf2 pathway, which can lead to upregulation of ROS production and cause oxidative stress in high concentration (Kim et al. 2010; Wang et al. 2020).

The most investigated pathway was the NF κ B signaling pathway, which is one of the major pathways of inflammation and immunity (Morgan & Liu 2011). This signaling pathway interacts with ROS production in many ways, and ROS plays a role in enhancing the transcription factors family that formed the NF κ B pathways (Takeda et al. 1999; Teramoto et al. 1999). This signaling pathway is not only upregulating the ROS production but also activated to promote the cell's survival from oxidative stress damage (Reuther-Madrid et al. 2002; Tang et al. 2002). These broad effects from the crosstalk of the NF κ B signaling pathway's proteins and ROS made this pathway the most investigated signaling pathway.

The second most investigated pathways in this study are Nrf2 and MAPK. The crosstalk between signaling proteins can create an intricate web of pathways of activation and deactivation of other proteins that lead to many biological processes. The Nrf2 and MAPK signaling pathways are examples of these crosstalks. The increasing level of ROS in oxidative stress conditions will activate the ERK-MAPK signaling proteins that lead to cell death (Wang et al. 2017). Because our body always tries to reach homeostatic conditions, with a high level of ROS, Nrf2 signaling pathways will also be activated to induce the upregulation of antioxidants, which will reduce the ROS level and eventually eliminate the oxidative stress condition (de Vries et al. 2008; J. Wang et al. 2017). With these opposite functions, MAPK and Nrf2 signaling pathways can be used as the biomarkers of the certain condition caused by oxidative stress.

5 CONCLUSION

Depending on the objectives of the studies, the methods and pathways investigated were different among the records. The signaling crosstalk intricate web of proteins that can be activated by ROS or in oxidative stress conditions in fibroblast lead to broad subjects that still need to be investigated. This also leads to the many methods to induce the ROS itself. It seems that the researchers still cannot determine the exact parameter for the oxidative stress condition in fibroblast, hence there is still not enough data to support the creation of oxidative stress as close as the natural pathologic condition, at least at the cell culture level.

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This systemic review was created as one of the outputs of the authors' research project. The authors declare no conflict of interest.

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