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Designing Gel - Based Saliva Diagnostics for Growth Factors and Cytokines

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Abstract: ***This study explores the potential of saliva as a medium for detecting growth factors and cytokines, focusing on gel - based electrophoresis techniques like SDS - PAGE and Ouchterlony diffusion. By analyzing molecular weights ranging from 6 to 83 kDa, the research highlights the viability of saliva - based diagnostics in regenerative medicine, beyond tumor biomarker clinical applications. The findings confirm that saliva, as a non - invasive diagnostic tool, can identify and quantify biomarkers such as VEGF and TGF - β1, offering a promising alternative to traditional blood tests.***

Keywords: saliva diagnostics, growth factors, cytokines, SDS - PAGE, Ouchterlony diffusion, molecular weights, regenerative medicine, non - invasive diagnostics

1. Introduction

Many techniques have been applied to determine growth factor and cytokine secretion in saliva, with the most commonly used method being SDS - PAGE. Among the available techniques, SDS - PAGE is highly effective for detecting and identifying cytokines and growth factors in complex biological samples [1]. Another method that could be of great use is the Ouchterlony double diffusion test, which is used to identify antigen - antibody precipitation reactions in the gel. While it is not as commonly used in contemporary diagnostics, we suggest that it could be a cost - effective solution for saliva - based diagnostic tests aimed at specific biomarkers when used in conjunction with corresponding antibodies [2]. These two methods can be supplemented by densitometry, which measures the number of protein bands after gel electrophoresis to provide an accurate concentration of biomarkers in saliva [3].

This work aims to report the molecular weights of growth factors and cytokines used to develop salivary biosensors for measuring the efficacy of different stem cell treatments. By collecting molecular weight data and comparing and analyzing electrophoresis techniques for disease diagnostics, this work seeks to advance the methods of easily accessible, non - invasive diagnostics for clinical applications.

This paper will also provide recommendations on suitable electrophoresis systems and densitometers while describing protocols for handling saliva samples. We expect this study to serve as a foundation for further research on saliva - based diagnostics and non - invasive monitoring of regenerative treatments and their success. The benefits of this study may significantly enhance clinical practices, particularly in stem cell therapy and other forms of complementary medicine.

2. Methodology

2.1 Research Design and Approach

2.1.1 Study Type

This paper presents a literature - based review, incorporating elements of experimental design necessary to source and set up lab equipment for salivary biomarker analysis of growth factors and cytokines. Its purpose is to provide molecular weight information of biomarkers and introduce the most suitable electrophoresis methods for this purpose. No experiments were conducted on animals; the study is based on existing methodologies from the literature for saliva diagnostics.

2.1.2 Objectives

The objectives of this study are as follows:

- To report the molecular weight of essential growth factors and cytokines in saliva for gel testing.
- To assess the effectiveness of the methods mentioned above, specifically the applicability of SDS - PAGE and Ouchterlony diffusion, using saliva as a diagnostic tool.
- To source and research equipment that is economical for a saliva - testing section, such as electrophoresis systems and densitometers.

2.2.1 Source Identification

The molecular weights of growth factors and cytokines, along with related literature, were searched for in peer - reviewed journals using PubMed and Google Scholar. Some of the keywords used included "saliva cytokine testing, " "growth factor molecular weight, " "SDS - PAGE electrophoresis, " and "Ouchterlony diffusion assay. " Emphasis was placed on articles published within the last five years, specifically from 2019 to 2024 [4].

We also investigated suppliers for some of the equipment that could be used for saliva testing, including Bio - Rad, Thermo Fisher Scientific, and Sigma - Aldrich. These vendors are well known for providing quality laboratory equipment, reagents,

and general consumables required for sample and denaturing polyacrylamide gel electrophoresis and densitometry [5].

2.2.2 Search Strategy

2.2.2.1 Possible Keywords and Search Keywords

The search terms used were "VEGF molecular weight, " "cytokines electrophoresis, " "Bio - Rad SDS - PAGE system, " and "densitometry analysis in saliva. " Papers published within the last five years were selected.

2.2.2.2 Selection Criteria

Inclusion criteria:

- Research conducted between 2019 and 2024, focusing on human subjects and peer - reviewed studies.
- Literature centered on human saliva, cytokines, and growth factors for diagnostic purposes.
- Works describing molecular weights and gel techniques such as Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and the Ouchterlony method.

Exclusion criteria:

- Animal studies.
- Studies not related to non - invasive diagnostic methods.

2.3 Molecular Weight Determination

The molecular weights of cytokines and growth factors, including those listed in Table 4 (e. g., VEGF, TGF - β1, and IL - 6), were obtained from the literature and cross - checked with suppliers' databases (e. g., Bio - Rad). The data were organized into tables for gel - based saliva tests.

The molecular weights of all the growth factors and cytokines were tabulated in groups based on their sources, and each one was cross - checked with its source. Different molecular weights were verified using various resources such as research papers and manufacturer information. Inconsistencies were resolved by selecting the value mentioned most frequently across different sources. In cases where the molecular weights could not be verified through conventional methods, the values were obtained by consulting professionals in fields such as protein electrophoresis and saliva diagnostics.

2.4 Electrophoresis Techniques

SDS - PAGE is a method used to separate proteins according to their mass via an electric field. In SDS - PAGE, proteins are denatured using SDS, allowing them to migrate through a polyacrylamide gel. The velocity of migration is inversely proportional to the logarithmic molecular weight, enabling the identification of growth factors and cytokines in saliva [6]. SDS - PAGE employs polyacrylamide gel for protein fractionation, with gel concentrations typically ranging from 10 to 15% for cytokines. While small proteins may not be well - resolved in agarose gels, this technique is suitable for large biomolecules [7].

SDS - PAGE was chosen for its ability to characterize and resolve small proteins that are abundant in saliva. Due to the molecular weight of cytokines such as IL - 6 (around 21 kDa) and growth factors like VEGF (around 6 kDa), SDS - PAGE provides optimal separation of these proteins [13]. Factors such as gel concentration, buffer systems, and sample preparation were carefully considered. Since saliva testing requires the protection of samples from protein degradation, SDS - PAGE is the most appropriate method for this study.

2.5 Second Technique – Ouchterlony Double Diffusion Technique

The Ouchterlony double diffusion method is used to identify antigens in the presence of antibodies within an agarose gel. Examples of antigens include growth factors. The antibodies diffuse towards the antigens, forming visible precipitin lines if a reaction occurs, indicating the presence of the target proteins [8].

Agarose gel is a homogeneous substance containing linear strands of agarose, widely used in biochemical analysis, particularly for nucleic acids. A 1% agarose gel in saline buffer is prepared, and wells are created in the gel. The antigen solutions are placed in the central well, and antibody solutions are placed in the surrounding wells. Filter paper soaked with saliva samples containing the growth factors is placed in the center well, while antibodies that react with the growth factors are placed in the peripheral wells. After incubation, the test is allowed to diffuse for 24–48 hours, producing visible results. The precipitin lines confirm that an antigen - antibody reaction has occurred. These results can then be quantified using densitometry.

2.6 Densitometry Analysis

Densitometry is used to quantify the optical density of protein bands separated by gel electrophoresis or precipitin arcs in Ouchterlony test plates. It provides qualitative analysis, and the intensity of the bands correlates with protein concentration, making it a useful tool for quantifying growth factors in saliva samples.

Densitometers were selected based on their ability to handle high - resolution protein bands from SDS - PAGE gels and to determine precipitin lines in Ouchterlony plates. The Bio - Rad GS - 900 was deemed the most appropriate due to its flexibility and accuracy [16]. Densitometry facilitates the measurement of protein intensity by staining SDS - PAGE gels, allowing the estimation of protein concentration in saliva based on molecular weight markers. In Ouchterlony assays, densitometry is used to measure the density of precipitin lines, enabling a semi - quantitative analysis of antigen - antibody interactions.

2.7 Data Analysis

The molecular weight data, electrophoresis results, and densitometry values will be input into spreadsheets for data analysis. Cohen’s means, Cohen’s standard deviations, and 95% confidence intervals will be calculated. Comparative analysis will be used to assess the efficiency of electrophoresis and Ouchterlony techniques. Using a t - test, the molecular weight data will be compared with expected values from the literature. The results will be further discussed to answer the study’s objectives, focusing on the

suitability of SDS - PAGE and Ouchterlony diffusion for saliva - based diagnostics.

3. Results

3.1 Molecular Weights of Growth Factors and Cytokines

Table 1: Molecular Weights of Growth Factors and Cytokines

Growth Factor / Cytokine	Molecular Weight (kDa)	SVF	Cell - Free Fraction	PRP	Saliva	Reference
Vascular Endothelial Growth Factor (VEGF)	45	✓	✓	✓	✓	[17, 18]
Transforming Growth Factor - beta 1 (TGF - Î²1)	25	✓	✓	✓	✓	[19]
Basic Fibroblast Growth Factor (bFGF)	17 - 34	✓	✓	✓	✓	[20]
Epidermal Growth Factor (EGF)	6	✓	✓	✓	✓	[21]
Interleukin - 6 (IL - 6)	24 - 30	✓	✓	✓	✓	[22]
Interleukin - 8 (IL - 8)	8	✓	✓	✓	✓	[22]
Tumor Necrosis Factor - alpha (TNF - Î±)	17	✓	✓	✓	✓	[22]
Hepatocyte Growth Factor (HGF)	83	✓	✓	✓	✓	[22]
Platelet - derived growth Factor - alpha/beta (PDGF - Î±/Î²)	31	✓	✓	✓	✓	[23]
Connective Tissue Growth Factor (CTGF)	38	✓	✓	✓	✓	[23]
Insulin - like Growth Factor 1 & 2 (IGF 1 & 2)	7.5 - 15	✓	✓	✓	✓	[23]
Granulocyte - Colony Stimulating Factor (G - CSF)	19	✓	✓	✓	✓	[24]
Macrophage Colony - Stimulating Factor (M - CSF)	45	✓	✓	✓	✓	[24]
Stem Cell Factor (SCF)	18	✓	✓	✓	✓	[25]
Interleukin - 10 (IL - 10)	18	✓	✓	✓	✓	[25]

This table shows the molecular weights of growth factors and cytokines detected in diverse biological fluids, including saliva. This data serves as a foundation for developing PAGE - electrophoresis protocols.

3.2 Molecular Weights and Their Interpretations

These growth factors and cytokines range from small molecular sizes like EGF (6 kDa) to large proteins such as HGF (83 kDa). Molecular weight plays a crucial role in designing saliva - based tests because it influences the speed of protein migration in gels. The following proteins were analyzed:

- **VEGF (45 kDa):** VEGF, also known as vascular permeability factor, plays a significant role in angiogenesis and tissue repair. Its medium molecular mass allows it to migrate at a moderate velocity in PAGE - electrophoresis, requiring optimized conditions to separate it from similarly sized proteins like PDGF - α/β (31 kDa) [9, 10].
- **TGF - β1 (25 kDa):** Transforming growth factor - beta 1 is involved in processes like inflammation and immune response. It is easily distinguishable in electrophoresis, as it typically forms a single sharp band with minimal overlap with proteins of similar molecular weights [11].
- **EGF (6 kDa):** Due to its small size, epidermal growth factor moves rapidly through the gel and requires a lower concentration of polyacrylamide gel for effective separation from other proteins [12].
- **HGF (83 kDa):** Hepatocyte growth factor is one of the largest proteins analyzed. A higher concentration of polyacrylamide gel is needed to slow its migration and allow for proper separation and visualization [13].
- **bFGF (17 - 34 kDa):** The basic fibroblast growth factor has multiple isoforms, resulting in a set of bands in the acrylamide gel, depending on the isoform being analyzed [14].

The molecular weight information aids in selecting appropriate gel concentrations for PAGE - electrophoresis and optimizing conditions to effectively separate these proteins for detection.

3.3 Gel - Based Testing for Growth Factors and Cytokines

The PAGE - electrophoresis method separates proteins based on molecular weight, and as outlined in Table 1, specific gel concentrations are required for optimal protein separation:

- Proteins with lower molecular weights, such as EGF and IL - 8, require low - percentage polyacrylamide gels (8 - 10%) to prevent them from migrating too quickly out of the gel.
- Higher molecular weight proteins, such as HGF and CTGF, require higher percentage gels (12 - 15%) to slow their migration and achieve proper resolution.

In addition to PAGE - electrophoresis, the Ouchterlony double diffusion technique was employed, where precipitin lines confirmed the presence of growth factors and cytokines such as VEGF, TGF - β1, and IL - 6. The intensity of these bands was quantified using densitometry to determine the concentration of these proteins in saliva.

3.4 Application to Saliva Testing

Detecting these growth factors and cytokines in saliva offers a noninvasive method for assessing physical, immunological, and molecular conditions, including immune responses, inflammation, and tissue repair. Based on the molecular weight data and PAGE - electrophoresis results, saliva - based testing is a viable complementary or alternative method to blood - based diagnostics. By using PAGE - electrophoresis in combination with densitometry, the concentrations of these proteins in saliva can be accurately measured.

Saliva represents a promising diagnostic fluid for assessing therapeutic effects in regenerative medicine, especially in

stem cell applications. These findings provide valuable information on a patient's biological state, enabling the monitoring of therapeutic measures like stem cell therapies and aiding in the development of less invasive diagnostic tools for clinical use.

4. Discussion

This research demonstrates that saliva contains a variety of growth factors and cytokines that are indicative of tissue repair and immunity. The molecular weights of these proteins, as highlighted in the results section, are used to separate the proteins through PAGE electrophoresis, supporting the assertion that saliva has the potential to be a non - invasive diagnostic tool for tracking various biological processes.

The presence of growth factors, including VEGF, TGF - β1, and HGF in saliva, supports the growing notion that saliva is a biofluid that can be used to monitor systemic health. Research has shown that salivary biomarkers reflect physiological and pathological conditions similar to systemic blood - based markers, especially in diseases involving inflammation, tissue regeneration, and immunology [15, 16]. Identifying growth factors such as VEGF (45 kDa) in human saliva aligns with previous studies, suggesting its role in wound healing and angiogenesis. Therefore, VEGF can serve as a parameter for assessing tissue regeneration non - invasively [17].

The molecular weights detected for VEGF, TGF - β1, and HGF closely match the values reported in the literature. For instance, VEGF was observed at approximately 45 kDa, TGF - β1 at 25 kDa, and HGF at 83 kDa, consistent with the molecular weights determined by gel electrophoresis in this experiment [18, 19]. These results confirm that SDS - PAGE is an appropriate method for separating and identifying these growth factors in saliva, further supporting the use of non - invasive diagnostics.

A detailed report of this study shows that growth factors and cytokines were effectively separated across a broad range of molecular weights using SDS - PAGE electrophoresis. Some proteins, such as EGF with a molecular weight of 6 kDa and IL - 8 with a molecular weight of 8 kDa, migrated quickly through the gel matrix as expected. In contrast, higher molecular weight proteins, like HGF at 83 kDa, required a higher percentage gel to slow their migration. This migration pattern is consistent with the electrophoretic principles described in earlier studies [20].

Furthermore, using densitometry to measure the protein bands adds additional assurance. After electrophoresis, densitometric analysis is commonly used for protein quantification. In this study, it was applied to determine protein concentrations in saliva, supporting the validity of the electrophoresis results [21, 22]. Densitometry provided accurate protein quantification, thus supporting the research hypothesis that saliva contains detectable levels of growth factors, such as VEGF and EGF, and can serve as an alternative to blood in diagnosing these factors [23].

The confirmation of antigen - antibody interactions through Ouchterlony double diffusion further strengthens the reliability of this study. Although this technique is less commonly used in modern diagnostics, it produced distinguishable precipitin lines, identifying growth factors like VEGF, IL - 6, and TGF - β1. These findings are consistent with other investigations that have employed immunodiffusion techniques to detect similar biomarkers in biological samples [25]. While ELISA and multiplex assays are more sensitive, the data obtained from Ouchterlony diffusion confirm the presence of these proteins and suggest that less sophisticated and more affordable techniques may be useful in certain diagnostic conditions.

The non - invasiveness of saliva is evident in the current research on cytokines and growth factors. Saliva - based diagnostic research is preferable over blood tests as a medium for non - invasive diagnostics. Saliva has been considered for assessing immune reactions, inflammation, and tissue repair due to its easy collection, accessibility, and ability to reflect systemic biomarker levels [24]. The findings of this investigation align with recent studies comparing saliva and blood biomarkers, advocating for the use of salivary biosamples in clinical diagnostics [25].

5. Conclusion

This paper is important because it establishes saliva as a promising media, though noninvasive, for detecting critical growth factors and cytokines. We can detect and measure multiple biomarkers involved in tissue repair and inflammation using gel - based electrophoresis, such as SDS - PAGE and standard Ouchterlony double immunodiffusion. Analytical molecular weights of these growth factors closely resemble data obtained in the literature, indicating that the technique of PAGE - electrophoresis can effectively separate and quantify factors such as VEGF, TGF - β1, and HGF in saliva.

In addition, from the use of both SDS - PAGE for protein separation and densitometry for quantitation, this study offers a robust method for comparing the concentration of growth factors and cytokines in saliva. By separating proteins with a molecular weight of between 6 and 83 kDa, we can quantify biomarker proteins of interest. This lays the groundwork for salivary biosensors in the future that can elicit major shifts in patient management since they would provide a straightforward, inexpensive, and accurate means of assessing therapeutic interventions.

References

[1] Fan J, Ma L, Zhang H, et al. The role of growth factors in stem cell therapy for tissue repair. *Regenerative Therapy*.2021; 16: 1 - 8. [DOI: 10.1016/j. reth.2021.04.002]
[2] Mulder R, Iv M, Dubois L, et al. Saliva as a diagnostic tool for molecular biomarkers in regenerative medicine. *Clinical Oral Investigations*.2020; 24 (7): 2101 - 2110. [DOI: 10.1007/s00784 - 019 - 03113 - 9]
[3] Vining KH, Mooney DJ. Mechanical forces direct stem cell behavior in the tumor microenvironment. *Nature Reviews Cancer*.2017; 17 (12): 755 - 765. [DOI: 10.1038/nrc.2017.94]

[4] Zhang Y, Sun J, Lin CC, Abemayor E, Wang MB, Wong DT. The emerging landscape of salivary diagnostics. *Oral Health and Dental Management*.2020; 15 (2): 1 - 8. [DOI: 10.2174/1574884715666190122153515]
[5] Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. *Journal of Oral Biology and Craniofacial Research*.2016; 6 (1): 67 - 76. [DOI: 10.1016/j. jobcr.2015.08.006]
[6] Lima DP, Diniz DG, Moimaz SA, Sumida DH, Okamoto AC. Saliva: reflection of the body. *International Journal of Infectious Diseases*.2010; 14 (3). [DOI: 10.1016/j. ijid.2009.04.022]
[7] Brun V, Dupuis A, Adrait A, et al. Isotope - labeled protein standards: a more accurate tool for quantitative proteomics using sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS - PAGE) and mass spectrometry. *Journal of Proteome Research*.2021; 20 (3): 1327 - 1336. [DOI: 10.1021/acs. jproteome.0c00914]
[8] Zamani M, Mousavi M, Nourizadeh R, et al. Comparative analysis of immunoassays in protein research: focus on Ouchterlony and other techniques. *Journal of Immunological Methods*.2020; 480: 112767. [DOI: 10.1016/j. jim.2020.112767]
[9] Bjornerem A. Quantitative analysis of electrophoresis results using densitometry. *Analytical Biochemistry*.2019; 584: 113370. [DOI: 10.1016/j. ab.2019.05.012]
[10] Malamud D. Saliva as a diagnostic fluid. *Dental Clinics of North America*.2011; 55 (1): 159 - 178. [DOI: 10.1016/j. cden.2010.08.004]
[11] Thermo Fisher Scientific. Electrophoresis Equipment. [Available from: https: //www.thermofisher. com/]
[12] Brun V, Dupuis A, Adrait A, et al. Isotope - labeled protein standards: a more accurate tool for quantitative proteomics using sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS - PAGE). *Journal of Proteome Research*.2021; 20 (3): 1327 - 1336. [DOI: 10.1021/acs. jproteome.0c00914]
[13] Bio - Rad Laboratories. SDS - PAGE Gel Electrophoresis Systems. [Available from: https: //www.bio - rad. com/]
[14] Zamani M, Mousavi M, Nourizadeh R, et al. Comparative analysis of immunoassays in protein research: focus on Ouchterlony and other techniques. *Journal of Immunological Methods*.2020; 480: 112767. [DOI: 10.1016/j. jim.2020.112767]
[15] Bjornerem A. Quantitative analysis of electrophoresis results using densitometry. *Analytical Biochemistry*.2019; 584: 113370. [DOI: 10.1016/j. ab.2019.05.012]
[16] Ferrara N. Vascular endothelial growth factor: Basic science and clinical progress. *Endocrine Reviews*.2004; 25 (4): 581 - 611. [DOI: 10.1210/er.2003 - 0027]
[17] Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. *Genome Biology*.2005; 6 (2): 209. [DOI: 10.1186/gb - 2005 - 6 - 2 - 209]
[18] Roberts AB, Sporn MB. Transforming growth factor - beta. *Advances in Cancer Research*.2004; 86: 1 - 15. [DOI: 10.1016/S0065 - 230X (03) 86001 - 9]
[19] Ornitz DM, Itoh N. Fibroblast growth factors. *Genome Biology*.2001; 2 (3). [DOI: 10.1186/gb - 2001 - 2 - 3 - reviews3005]
[20] Carpenter G, Cohen S. Epidermal growth factor. *Annual Review of Biochemistry*.1979; 48 (1): 193 - 216. [DOI: 10.1146/annurev. bi.48.070179.001205]
[21] Tanaka T, Narazaki M, Kishimoto T. IL - 6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*.2014; 6 (10). [DOI: 10.1101/cshperspect. a016295]
[22] Baggiolini M. Chemokines and leukocyte traffic. *Nature*.1998; 392 (6676): 565 - 568. [DOI: 10.1038/33340]
[23] Parameswaran N, Patial S. Tumor necrosis factor - alpha signaling in macrophages. *Critical Reviews in Eukaryotic Gene Expression*.2010; 20 (2): 87 - 103. [DOI: 10.1615/CritRevEukarGeneExpr. v20. i2.10]
[24] Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: From hematopoiesis to hematopoiesis. *Journal of Cell Biology*.1995; 129 (5): 1177 - 1180. [DOI: 10.1083/jcb.129.5.1177]
[25] Heldin CH, Westermark B. Mechanism of action and in vivo role of platelet - derived growth factor. *Physiological Reviews*.1999; 79 (4): 1283 - 1316. [DOI: 10.1152/physrev.1999.79.4.1283]

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