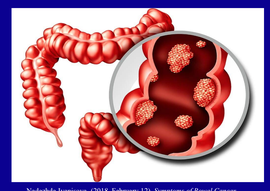
An Innovative Approach To Reduce Biofilm Formation & Progression: Evaluating the Impact of Antimicrobial Compounds on Thioredoxin-A in Biofilm for Colorectal Cancer Treatment

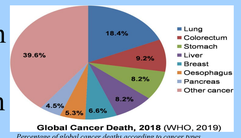
Colon Cancer Awareness Month: John Vizuete, MD, MPH: Gastroenterologist. (n.d.). Www.johnvizuetemd.com. https://www.johnvizuetemd.com/blog/colon-cancer-awareness-month



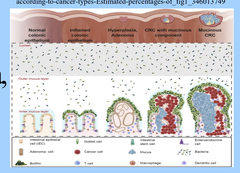
Nadezhda Ivanisova. (2018, February 12). *Symptoms of Bowel Cancer*. Booking Health. Hospital and the Best Health Care Booking Service in the World; Booking Health. https://bookinghealth.com/blog/diagnoses-and-treatment/diagnosis-and-treatment/332276-symptoms-of-bowel-cancer.html

Introduction/ Background

- Cancer is one of the major reason of death in today's world
- Colorectal Cancer develops from certain polyps or growths in the inner lining of the large intestine.
- A biofilm is a community of microorganisms, such as bacteria, that adhere to a surface and each other, forming a protective matrix.
- Thioredoxin-A (Trx-A) is a multifunctional protein ubiquitously found in the human body. Trx-A plays an important role in various cellular functions such as maintenance of redox homeostasis, proliferation, and DNA synthesis.



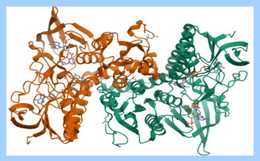
Percentage of global cancer deaths according to cancer types Estimated... (2024). ResearchGate. https://www.researchgate.net/figure/Percentage-of-global-cancer-deaths-according-to-cancer-types-Estimated-percentages-of_fig1_346013749



Li, S., Pepplenbosch, M. P., & Smits, R. (2019b). Bacterial biofilms as a potential contributor to mucinous colorectal cancer formation. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1872*(1), 74–79. https://doi.org/10.1016/j.bbcan.2019.05.009

Purpose/ Research Question

Purpose: Colorectal Cancer ranks as the fourth leading cause of cancer-related deaths. Often covered in biofilms, right sided tumors are deadly and often identified in advanced stages. Biofilms cloak the tumor, providing a protective barrier against any external pressures such as treatments. Thioredoxin A(TrxA) is a key protein in bacterial redox regulation that has a complex and context-dependent impact on biofilm formation so the goal of this experiment is to find an antimicrobial intervention that is most effective in inhibiting Trx-A.



Data, P. (2017). *RCSB PDB - 2NVK: Crystal Structure of Thioredoxin Reductase from Drosophila melanogaster*. Rcsb.org. https://www.rcsb.org/structure/2NVK

Many studies have shown that Thioredoxin-A has a huge impact on biofilm progression such as maintain redox balance and helping the bacteria survive oxidative bursts from immune cells

Research Question: Which antimicrobial intervention is the most effective against Thioredoxin-A?

Hypothesis

Hypothesis: If a specifically designed ShRNA strain and other antimicrobial interventions are tested against Trx-A in a computer simulation, then the ShRNA will be most effective at reducing Trx-A activity, because ShRNA is a type of RNA molecule that can silence gene expression in human cells whereas the other substances are external components and may not impact Trx-A as much.

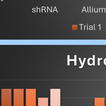
Methodology

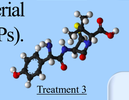
Variables

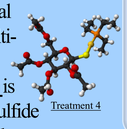
Independent Variable	Dependent Variable	Controlled Variables
ShRNA, Allicin, Oxacillin, Auranofin, and Dithiothreitol	Trx-A enzymatic activity inhibition- the response of the simulation to the different treatments	Trx-A Protein Structure and Simulation environment parameters

Antimicrobial Interventions:

Molecular Approach – Short hairpin RNA (shRNA) is effective for silencing genes by triggering the breakdown of target mRNA.
Organic Approach – Allicin found in Allium Sativum (Garlic) has been shown to inhibit thioredoxin reductase (TrxR), an essential enzyme in bacterial redox systems. The inhibition of this enzyme can lead to antibacterial effects, especially in biofilm-prone bacteria.
Antibiotics- Oxacillin is a B-lactam antibiotic that targets bacterial cell wall synthesis by inhibiting penicillin-binding proteins (PBPs). This action disrupts cell wall construction, effectively killing bacteria.
Targeting Bacterial Systems – Auranofin is an antimicrobial agent, effective against Gram-positive bacteria, including multi-drug-resistant strains.
Breaking of chemical composition – Dithiothreitol (DTT) is often used in biochemical research for its ability to reduce disulfide bonds. Its antioxidant action prevents the formation of disulfide bridges within proteins, preserving them in a reduced state, which can help disrupt cellular processes in microbes under certain experimental conditions.


Treatment 2


Treatment 3


Treatment 4


Treatment 5

Procedures

1. Gather necessary materials (Computer and stable internet connection) and download the 3d structure file of the Trx-A protein in PDB Format along with the 3d files of each intervention being tested.

2. Open the Trx-A protein structure in the docking software, then prepare the protein by removing unnecessary molecules, such as water.

3. Prepare the 3D structures of each ligand by loading them into the docking software and optimizing the structures required, which includes assigning proper charges to ensure accurate docking results.

4. Configure the docking parameters in the software by selecting Trx-A as the target protein and each ligand at a time. Then define a docking grid around the active site of Trx-A to focus the simulation on the area where binding is likely to occur, adjusting parameters like the binding mode and search algorithm

5. Start the docking simulation for each compound to determine critical data such as binding affinity, RMSD (Root Mean Square Deviation), and the number of hydrogen bonds formed between each compound and Trx-A, which will indicate the strength and stability of each bond.

6. Analyze the results by comparing the binding affinity, RMSD values, and the number of hydrogen bonds formed, to assess which compound demonstrates the strongest and most stable binding to Trx-A, suggesting its potential effectiveness in blocking Trx-A's activity in Biofilms.

File:R-allicin-3D-balls.png - Wikimedia Commons. (2007, August 8). Wikimedia.org. https://commons.wikimedia.org/wiki/File:R-allicin-3D-balls.png
Treatment 2

Sign in - Google Accounts. (2025). Wikimedia.org. https://commons.wikimedia.org/wiki/File:Amoxicillin-3D-balls.png
Treatment 3

File:Auranofin-from-xtal-3D-balls.png - Wikipedia. (2009, February 12). Wikipedia.org. https://en.m.wikipedia.org/wiki/File:Auranofin-from-xtal-3D-balls.png
Treatment 4

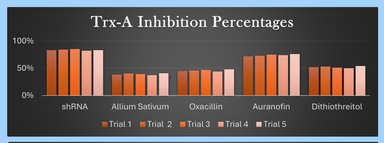
File:Dithiothreitol-3D-balls.png - Wikimedia Commons. (2011, May 31). Wikimedia.org. https://commons.wikimedia.org/wiki/File:Dithiothreitol-3D-balls.png
Treatment 5

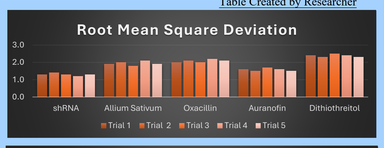
Results

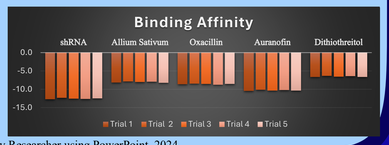
Treatment	Description	Trx1 Inhibition	RMSD	H-Bonds	Binding affinity
ShRNA	ShRNA gradually demonstrated an inhibitory effect, with a delayed onset compared to other treatments. It showed a targeted approach, binding specifically to Trx-A related RNA sequences.	83%	1.3	4.8	-12.5
Allicin	Allicin found in Allium Sativum extract exhibited mild inhibition of Trx-A, with weaker binding than synthetic compounds	39%	1.9	3.2	-8.0
Oxacillin	Oxacillin had moderate interactions with Trx-A. It appeared less effective at blocking the active site fully.	46%	2.1	2.2	-8.5
Auranofin	Auranofin displayed a strong binding affinity to the active site of the Thioredoxin-A, indicating high potential as an inhibitor.	74%	1.6	1.6	-10.3
Dithiothreitol	Dithiothreitol showed variable results in its simulated interaction, with some affinity but weaker then Auranofin	52%	1.4	1	-6.5

Table Created by Researcher

Quantitative data showed a clear pattern of binding affinities, with shRNA demonstrating the strongest inhibition across all trials. This suggests its highly specific interaction with Trx-A, potentially due to its design to target the gene directly. Auranofin, while less potent than shRNA, maintained consistent inhibition, indicating its potential as a synthetic therapeutic. On the other hand, Dithiothreitol and Oxacillin displayed weaker binding affinities and higher variability in their effects. Interestingly, Allium sativum exhibited moderate inhibition, likely due to its natural bioactive compounds, but its results varied across trials, reflecting the complexity of using plant-based interventions.







All Graphs Created by Researcher using PowerPoint, 2024

Abstract

In today's world Colorectal Cancer ranks as the fourth leading cause of cancer-related deaths. Often covered in Biofilms, right sided tumors are deadly and often identified in advanced stages. Biofilms are complex bacterial communities that cover the tumor providing protection from external pressures. Furthermore, many studies show that Thioredoxin-A (Trx-A) has a huge impact on Biofilm formation and progression so reducing Trx-A activity might help weaken biofilms and improve treatment. This experiment investigates which of the five antimicrobial compounds is most effective against Trx-A.

The tested compounds are shRNA, Auranofin, Oxacillin, Allium Sativum, and Dithiothreitol. The purpose is to see which compound binds best to Trx-A. Using computer simulations, each compound was virtually "docked" to measure binding affinity, Root Mean Square Deviation, and the number of hydrogen bonds created. Results showed that shRNA had the highest binding affinity and most stable bond, followed by Auranofin, while the Allicin in Allium Sativum had the lowest scores in all categories. ShRNA also created the most Hydrogen bond with Trx-A, suggesting it forms a strong stable interaction that could block Trx-A effectively. In conclusion, these findings suggest shRNA, with its strong and stable binding to Trx-A, might be the most promising choice for future research into treatments targeting biofilm-associated infections in cancer. Further studies could validate these results in real-life lab conditions as this project aims to help Colorectal Cancer in the future.

Image created by Researcher using Canva, 2025

Conclusion

This experiment found the shRNA was the most effective compound for targeting the protein Thioredoxin-A(Trx-A) in Biofilms, out of all five tested treatments. ShRNA had the strongest and most stable bond with Trx-A. This strong bond can help against Trx-A because the stronger a compound binds, the more it can interfere with the Trx-A's ability to support biofilm formation and growth. This is important because biofilms protect harmful bacteria making tumors harder to treat. Furthermore, Auranofin also showed good results but was not as effective as ShRNA. The other ligands Allicin, Oxacillin, and Dithiothreitol had weaker bonds with Trx-A. These results suggest that computer simulations can be a helpful way to test which compounds might work best before trying them in real life experiments.

Future Work

This project can be expanded by exploring additional compounds or natural substances that may further inhibit Trx-A activity, potentially identifying new therapeutic agents for treating biofilms. Further research could also investigate the long-term effects of these inhibitors on bacterial growth and biofilms persistence, offering a broader understanding of their potential in combating chronic infections. Additionally, scaling up this research to include clinical trials would help validate the effectiveness of these compounds in humans, making it possible to develop targeted therapies, By continuing to refine the methods and expanding, this project could contribute to developing innovative treatments for Colorectal Cancer. In real-world applications, these findings could help treat many problems paving the way for more personalized and sustainable healthcare solutions.