TNF-α Concentration Curve Effect on Oral Squamous Cell Carcinoma In Vitro

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Introduction

- Cancer involves abnormal cell growth that can spread to other parts of the body.
- Head and neck cancer refers to cancers that originate in the tissues of the head and neck region, including the throat, mouth, nose, sinuses, and salivary glands.
- Oral cancer typically includes cancers of the lips, tongue, gums, mouth, and the lining inside the cheeks.
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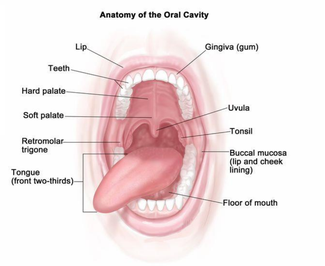
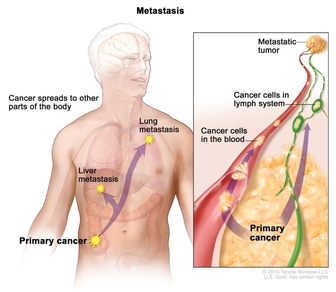
- Factors that can increases risk include tobacco and alcohol use, human papillomavirus (HPV), and exposure to certain chemicals or radiation.



- Metastasis is the spread of cancer cells from the site of the original tumor to other parts of the body.
- Epithelial-to-Mesenchymal Transition (EMT) is a biological process where epithelial cells lose their characteristics and acquire properties similar to mesenchymal cells.



- Inflammation can induce Epithelial-to-Mesenchymal Transition (EMT) in cells, promoting tissue remodeling and repair.
- **TNF-α** is a cytokine produced primarily by activated macrophages.
  - It plays a central role in inflammation and immune response regulation.
  - TNF-α is involved in initiating and promoting inflammation by inducing cytokine production, leukocyte recruitment, and vascular endothelial cell activation.
  - It has both pro-inflammatory and anti-inflammatory effects depending on the context and concentration.
  - In cancer, TNF-α can have both anti-tumor and pro-tumor effects depending on the specific tumor microenvironment and context of its expression.

Cell Lines

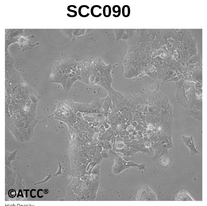
Oral Squamous Cell Carcinoma are a type of cancer that originates in squamous cells lining the oral cavity and mucosal surfaces.

Characteristics:
- Most common oral cancer, comprising about 90% of cases.
- Often begins as a white patch (leukoplakia) or red patch (erythroplakia) that doesn't heal.

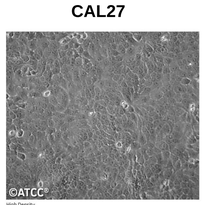
CAL27
- Oral; tongue cancer
- BSL1
- White, 56, male
- Tumorigenic
- Epithelial, polygonal with a highly granular cytoplasm

UPCI:SCC090
- Oral; tongue cancer
- BSL2
- White, 46, male
- Positive for Human Papilloma Virus (HPV)

SCC090



CAL27



Methods

Wound Healing Assay

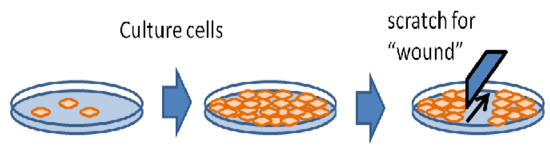
A migration assay, also known as a wound healing assay, is a lab technique used to study cell movement, migration, and reaction.

Purpose:
- The assay is designed to observe and quantify how cells migrate into an empty space created in a cell culture. This mimics processes like wound healing in vivo and helps study cell migration in response to various stimuli or conditions.

Procedure:
- Cell Culture: Cells of interest are cultured until they reach confluence.
- Creating the Wound: A uniform scratch or gap ("wound") is made across the cell layer using a sterile tool like a pipette tip. This creates an empty space where cells have been removed.

Data Collection:
- Imaging: Images are taken at each time point to track the movement of cells into the wound area.
- Analysis: The extent of wound closure (gap reduction) and the rate of cell migration are quantified using image analysis software or manually by measuring the remaining gap width over time.

Applications:
- Research: Studying the effects of various factors (growth factors, drugs, genetic modifications) on cell migration.
- Drug Development: Screening potential drugs that affect cell migration, relevant in fields such as cancer research where cell motility is crucial.



MTT Assay Proliferation/Viability Assay

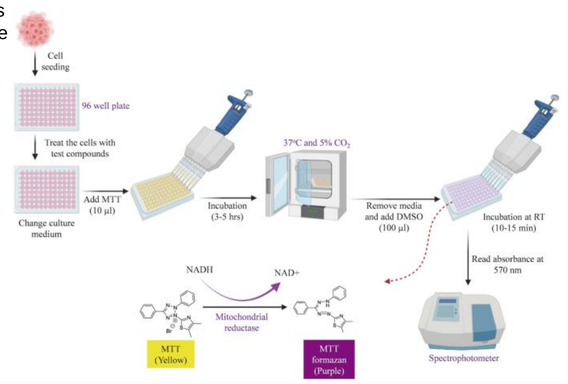
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Purpose: Assess cell viability and proliferation.

Procedure:
- Treat cells with compounds/drugs of interest.
- Add MTT solution to cells.
- Incubate to allow MTT to be metabolized.
- Add a solubilization solution (e.g., DMSO) to dissolve formazan crystals.
- Measure absorbance at appropriate wavelength (typically 570 nm).

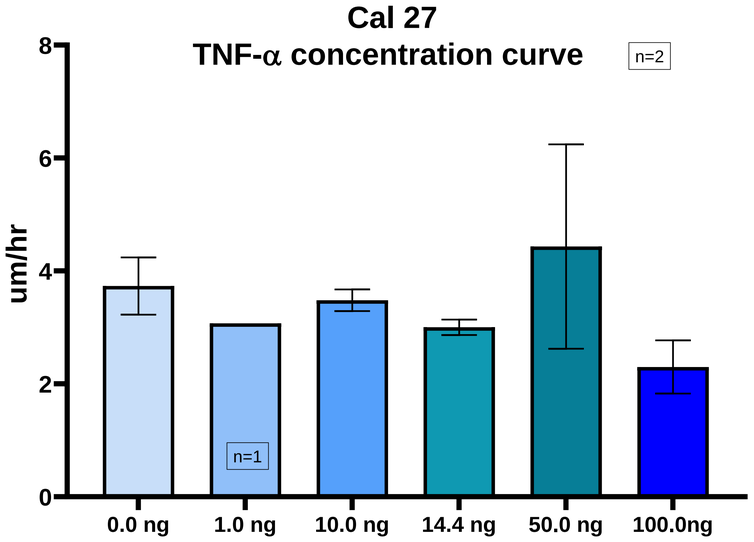
Data Collection:
- Higher absorbance correlates with more metabolically active cells.
- Lower absorbance indicates fewer viable cells or reduced metabolic activity.

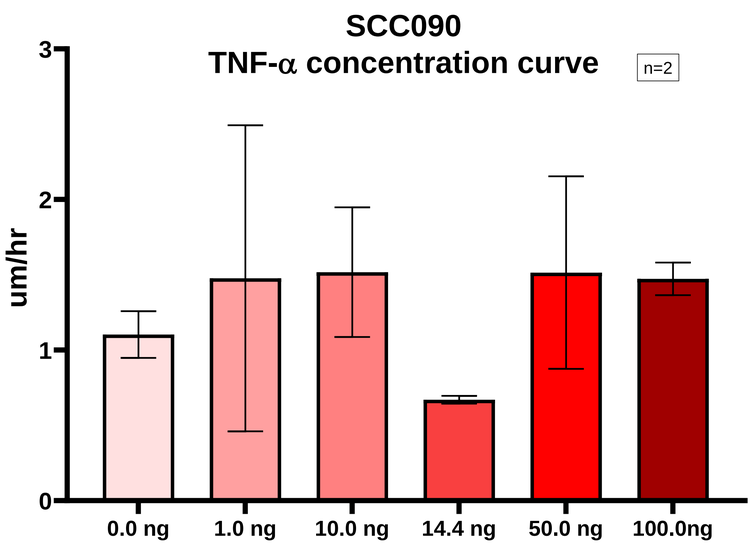
Applications:
- Drug screening and toxicity testing.
- Assessing cell proliferation rates.
- Evaluating cytotoxicity of compounds.



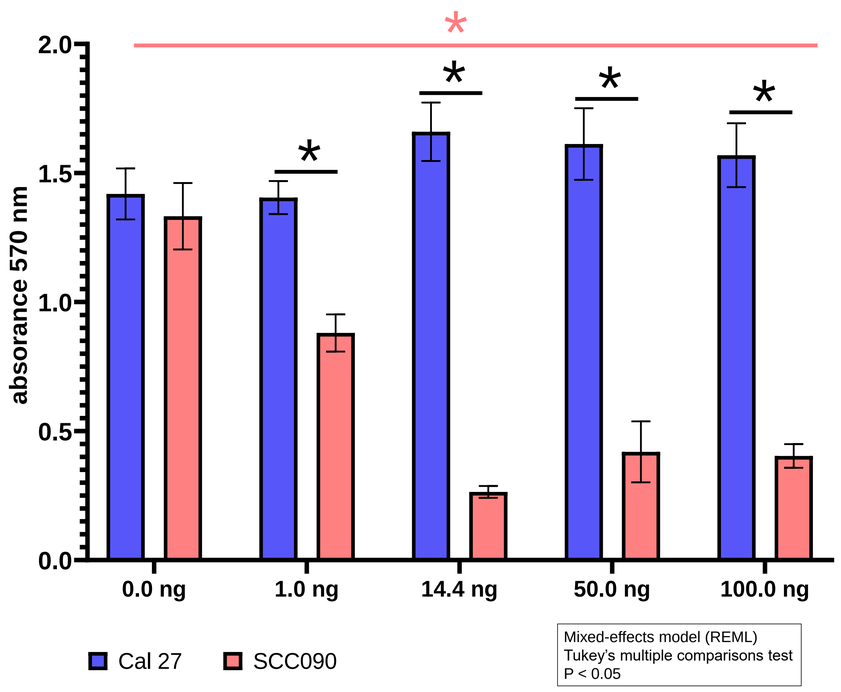
Results

Wound Healing Assay





MTT Assay Cal 27 and SCC090



Conclusion

- Over a 24-hour period, TNF-α concentration had no significant effect on Cal 27 cells, but significantly lowered proliferate of SCC090 at concentration: 0.0, 1.0, 14.4, 50.0,100.0 ng.
- Preliminary experiment on TNF-α effect on motility rate of Cal 27 and SCC090 showed an overall lower rate of motility of SCC090 than Cal 27 over a 24-hour period with no statistical analysis performed due to low number of samples.

Future Directions

- Repeat the TNF-α concentration curve motility assay to increase sample size.
- Replicate experiments using nicotine as condition challenge.
- Replicate experiments with bacteria metabolites that are linked to the promotion of inflammation and tumor growth.

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