# The Effect of Vitamin C on HeLa Cell Proliferation

James Dinger, Allysa Steinhaus, Charleen Horn, Isabella Dory
Faculty Advisor: Dr. Bergerson, Concordia University St. Paul

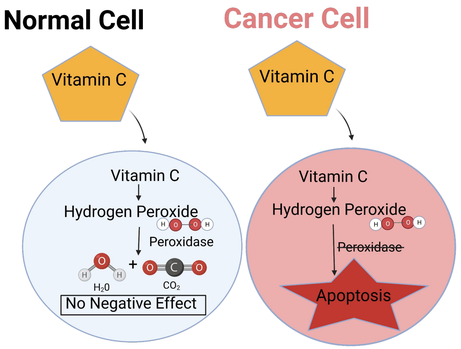
## Abstract

In this study, we investigated the impact of vitamin C on cervical cancer cells at varying concentrations. After adding vitamin C reagent to plated HeLa cells, we performed cytotoxicity assays to measure cell viability. We hypothesized that higher concentrations of vitamin C would lead to lower cell viability, as vitamin C is predicted to cause cell death in cancer cells. Our results indicated that higher concentrations of vitamin C do not necessarily correlate with lower cell viability of cervical cancer cells.

## Background

- Vitamin C has been shown to have numerous beneficial effects on the body, specifically on the maintenance of healthy cells.
- Studies have been done relating the risk of HPV infection to low intakes of vitamin C[1] It has been shown that a daily intake of 50 mg/day of vitamin C could lead to a decrease in cervical neoplasia that leads to cervical cancer[1].
- Another study has been done working with cervical cancer patients and treating them with cisplatin and vitamin C to induce cell apoptosis.
- It was concluded that vitamin C can reduce HPV infection and inhibit the development of cervical cancer[1].
- Upon reading literature, we found that high concentrations of vitamin C ranged from 0.3-20 mmol/L[2]. Based on this scale, we chose a lower concentration (0.5 mmol), medium concentration (0.9 mmol), and high concentration (2.73 mmol) to test on the cervical cancer HeLa cells .

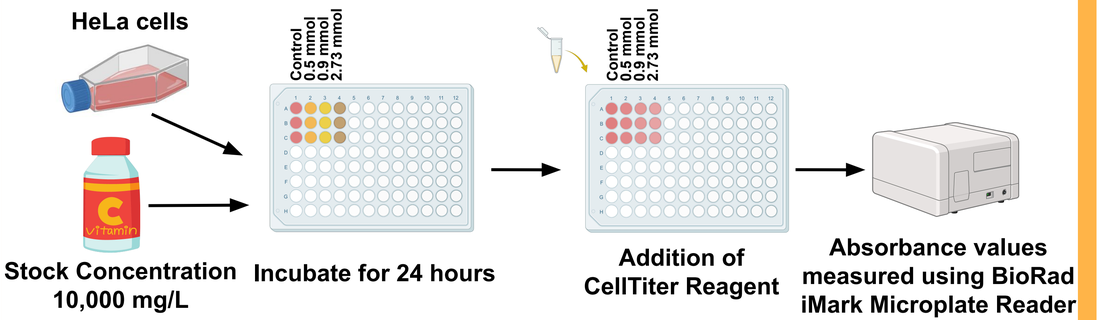
## Proposed Mechanism of Vitamin C




- Vitamin C is typically an antioxidant which means it helps prevent cell death[3].
- At high concentrations, vitamin C acts as a pro-oxidant meaning it leads to cell death. It is able to directly deliver hydrogen peroxide to cancer cells[4].
- Hydrogen peroxide is an unstable molecule that causes cell damage, and can eventually lead to cell death[5].

## Methods

- Three different concentrations of Vitamin C were added (0.5 mmol, 0.9 mmol, and 2.73 mmol) to plated HeLa cells.
- The cells were incubated for 24 hours and a Cytotoxicity Assay was performed.
- This experiment was performed in triplicate, four times




## Results

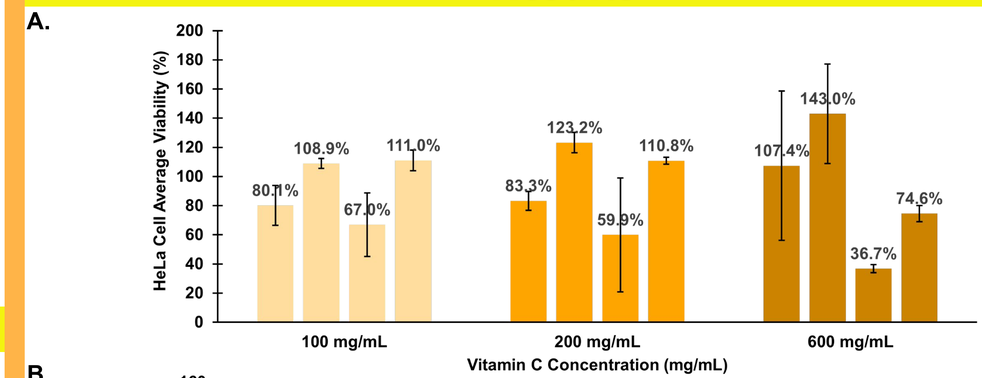



**FIGURE 1. Quantification of HeLa cell viability growing in different vitamin C concentration as percentages.** HeLa cells were plated with control, 100mg/mL, 200mg/mL, 600mg/mL added vitamin C in 200µL wells in triplicates for 24 hours. A cytotoxicity assay was performed after incubation to test cell viability.

- Cell viability is not found to decrease or increase on average as vitamin C concentration increases.
- Cell viability with 100mg/mL vitamin C added showed an increase twice and decrease twice
- Cell viability with 200mg/mL vitamin C added showed an increase twice and decrease twice
- Cell viability with 600mg/mL vitamin C added showed an increase twice and decrease twice

## Discussion

- Vitamin C did not have an impact on HeLa cell viability as there was no correlation between growing HeLa cells with vitamin C.
- Data supports neither an increase nor decrease of HeLa cell viability.
- Reasons for the inconsistent data could be due to execution attributed to human errors, while a misestimation of the variable vitamin C concentration would also be a possibility.
- Vitamin C concentrations used in this experiment are significantly lower to previous studies. The used concentrations could have been too low for causing a change in cell viability, and could have contributed to visibly inconsistent data.
- Using low vitamin C concentrations could be a reason why neither an increase nor decrease in cell viability can be seen.
- The vitamin C concentrations did not differ significantly from one another (0.5mmol, 0.9 mmol, 2.73mmol) causing an insubstantial change in the environment, which may have contributed to not seeing an increase or decrease in cell viability.
- Vitamin C and medium may have not been mixed well enough in the wells when plating, thus creating an inconsistent environment that affects cell viability.
- Due to non-matching data and since no trend is apparent, the hypothesis can neither be confirmed nor disproved.

## Future Experiments

- For future experiments, we would use higher concentrations of vitamin C as this was a limitation in the study due to a restricted amount of resources.
- In addition, we would use greater differences between the amounts of vitamin C concentrations to see if there were significant changes in cell viability per increment.
- For future references, we will do trypan blue cell staining and hemocytometer counting to make sure the same number of cells are being used in each trial to try and best replicate each trial as we struggled getting consistent data.

## References

1. A. Ono *et al.*, The preventive effect of dietary antioxidants on cervical cancer development. *Medicina*. **56**, 604 (2020).
2. A. Mussa *et al.*, High-dose vitamin C for cancer therapy. *Pharmaceuticals*. **15**, 711 (2022).
3. G. R. Buettner, The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and Ascorbate. *Archives of Biochemistry and Biophysics*. **300**, 535–543 (1993), doi:10.1006/abbi.1993.1074.
4. G. Calcutt, The formation of hydrogen peroxide during the autoxidation of ascorbic acid. *Experientia*. **7**, 26–26 (1951), doi:10.1007/bf02165477.
5.S. Park *et al.*, L-ascorbic acid induces apoptosis in acute myeloid leukemia cells via hydrogen peroxide-mediated mechanisms. *The International Journal of Biochemistry & Cell Biology*. **36**, 2180–2195 (2004), doi:10.1016/j.biocel.2004.04.005.