Investigating the Effects of Glucosamine and N-Acetylglucosamine on CHO-K1 Cell Behaviors

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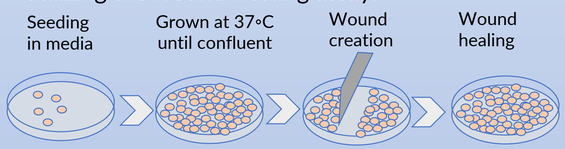
BACKGROUND:

- Glucosamine (GlcN) and N-Acetylglucosamine (GlcNAc) are glucose metabolites of the hexosamine biosynthesis pathway (HBP).
- HBP is involved in many cellular processes such as protein glycosylation, signal transduction, and gene expression regulation.
- Protein glycosylation is vital for substrate specificity and protein-protein recognition in membrane proteins and receptors.
- GlcN and GlcNAc are both used interchangeably to study the HBP. While GlcN is significantly more studied, very few studies focus on GlcNAc.

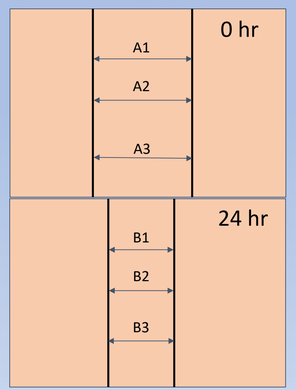
METHODS

- We studied Chinese hamster ovary K1 (CHO-K1) utilizing the wound-healing assay:



- Each well was coated with 1% gelatin to mimic the extracellular matrix (ECM).
- Cells were treated with different concentrations of GlcN or GlcNAc in growth media, supplemented with either high (4.5 g/L) or low glucose (1.5 g/L) concentration.
- The wound size was monitored at defined time intervals and measured using ImageJ software

DATA ANALYSIS:



Wound size at 0 hr (mm):
A = (A1 +A2 + A3)/3

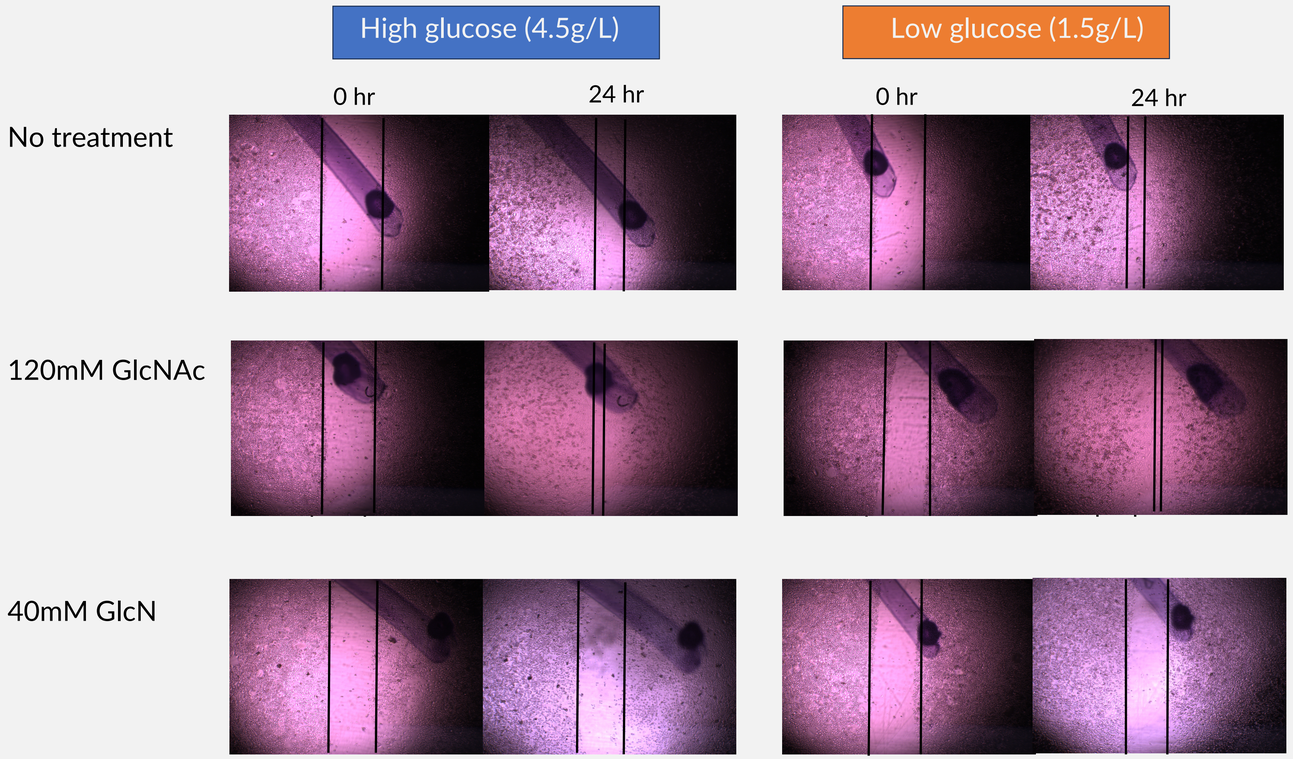
Wound size at 24 hr (mm):
B = (B1 + B2 + B3)/3

Reduction in wound size after 24h (mm):
D = A - B

Migration rate (reduction per hour) (mm/hr):
V = D/24

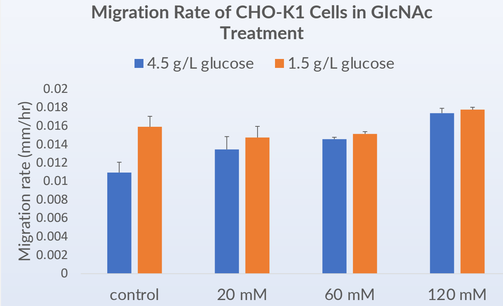
Exogenous sugars, specifically Glucosamine and N-Acetylglucosamine, have **distinct effects** on cell migration and morphology

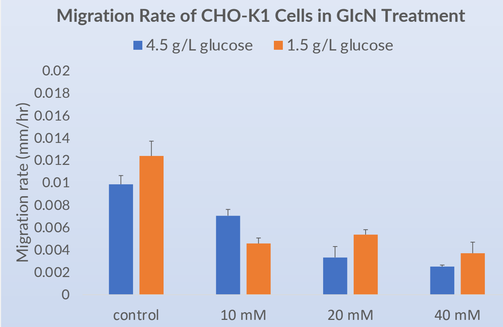
CHO-K1 cell scratch distance – 4.5 g/L glucose



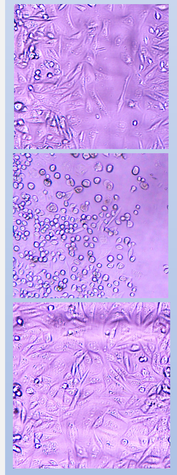
Cell migration over time under no treatment, 120 mM GlcNAc, and 40 mM GlcN concentrations. The dark vertical lines on the images indicate the boundary of the wound captured after a specific time interval. Migration distance was measured by subtracting the wound size at 0-hour mark to the wound size at 24-hour mark.

RESULTS





CHO-K1 Cells 24 hours after scratching - High glucose (4.5 g/L)



No treatment
- Moderate cell-to-cell adhesion
- Strong ECM attacthent

40 mM GlcN treatment
- Weak cell-to-cell adhesion
- Weak ECM attachment

120 mM GlcNAc treatment
- Strong cell-to-cell adhesion
- Strong ECM attachment

DISCUSSION

- GlcN and GlcNAc have distinct influences on cell behaviours and morphology, pointing to alterations in cytoskeleton and membrane protein expression .
- The influence of GlcN and GlcNAc on cells may be affected by variations in glucose concentration.
- Significant implications for immune response, vascular health, diabetes, as well as cancer metastasis.

REFERENCES

Dhillon, P. K., Li, X., Sanes, J. T., Akintola, O. S., & Sun, B. (2017). Method comparison for analyzing wound healing rates. *Biochemistry and Cell Biology*, *95*(3), 450–454. https://doi.org/10.1139/bcb-2016-0163

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