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Research Article

Effects of single and intermittent glucose exposure on hyaluronic acid, heparan sulfate, and syndecan expression in HUVECs cells

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ABSTRACT
Objectives: This study aims to investigate 1) the effect of incubation duration on changes in HUVECs cell glycocalyx expression, 2) the impact of glucose dose on changes in HUVECs cell glycocalyx expression, and 3) the comparison of single and intermittent glucose doses on changes in HUVECs cell glycocalyx expression.
Methods: After confluence, the HUVECs were divided into three steps to study. On the first steps, HUVECs were divided into control and duration of incubation of 12-hours; 24-hours; 48-hours and 72-hours. On the second step, HUVECs were classified according to the glucose concentration exposure (0 mM; 5 mM or 22 mM) for 72-hours of incubation. On the third step. HUVECs were divided into single and intermittent doses (for 72-hours of incubation).
Results: The expression of hyaluronic acid, heparan sulfate, and syndecan was analyzed by laser scanning confocal microscope.
Conclusions: In conditions without glucose exposure and physiological glucose exposure, glycocalyx has a dynamic expression. High glucose exposure eliminates the dynamics of hyaluronic acid and syndecan expression. Single-dose exposure triggers a decrease in hyaluronic acid expression compared to intermittent exposure, and this is the opposite of heparan sulfate exposure.

Keywords: glycocalyx, endothelial cells, single dose, intermittent dose.

INTRODUCTION

Endothelial cells are complex endocrine and paracrine organs that play a physiological role in the modulation of vascular tone (vasoconstriction and vasodilation), hemostasis, growth regulation and differentiation of vascular smooth muscle cells, and inflammatory modulation (Hossain, Kawar and El Nahas, 2007). Diabetes mellitus is a chronic endocrine disease due to relative or absolute insulin deficiency (Kristensen et al., 2016). Cardiovascular complications are a life threat to people with diabetes mellitus, and their progress is mostly determined by the status of hyperglycemia (Dalan et al., 2014; Fox et al., 2015). Hyperglycemia triggers dysfunction, damage, and death of endothelial cells and acceleration of atherosclerosis as a basis for cardiovascular complications (Deguchi and Miyazaki, 2010; Georgescu et al., 2011; Bammert et al., 2017).

Endothelial cell function is determined by the structure of the cytoskeleton and glycocalyx (Fels et al., 2014). Glycocalyx consists of glycosaminoglycans, proteoglycans, and glycoproteins (Barakat, 2008; Fu and Tarbell, 2013; Sieve, Münster-Kühnel and Hilfiker-Kleiner, 2018). Hyaluronic acid is a linear glycosaminoglycan that is chemically composed

of N-acetylglucosamine and D-glucoronic acid through b-1.3 glycosidic bonds and disaccharide units which are bound to b-1.4 glycosidic (Huang, Chen and Chen, 2018). Heparan sulfate is linear polysaccharides arranged by N-acetylated or N-sulfonated glucosamine units and uronic acid. Heparan sulfate occupy 50% -90% glycosaminoglycan (Tarbell and Pahakis, 2006; Reitsma et al., 2007; Saito, 2015). Syndecan-1 transmembrane is a core protein of glycocalyx. Hyaluronic acid chains and heparan sulfate attach to syndecan-1 and are located on the lumen surface of the glycocalyx network (Saito, 2015).

Two things underlie the emergence of complications of diabetes mellitus, the degree of hyperglycemia, and hyperglycemia fluctuations (Home, 2005). Acute hyperglycemia triggers glycocalyx degradation. Exposure to high glucose levels triggers syndecan shedding. Diabetes mellitus triggers an increase in hyaluronic acid plasma as well as shedding syndecan (Nieuwdorp et al., 2006; Broekhuizen et al., 2010). Glucose fluctuations (peak hyperglycemia and hypoglycemia) will trigger the production of reactive oxygen compounds and cellular apoptosis, which are more massive than persistent high sugar levels (Ricks et al., 2012; Quincozes-Santos et al., 2017). In HUVECs cells exposed to glucose 5.5 mM and 22 mM alternatively, reactive oxygen compounds will be formed, which are more significant than constant exposure (Quincozes-Santos et al., 2017). To the best of our knowledge, the effects of hyperglycemia fluctuations on glycocalyx are unclear. Therefore, this study aims to investigate 1) the impact of incubation duration on changes in HUVECs cell glycocalyx expression, 2) the effect of glucose dose on changes in HUVECs cell glycocalyx expression, and 3) comparison of single and intermittent glucose doses on changes in HUVECs cell glycocalyx expression.

Methods

Culture cells

HUVEC cells were purchased from ATCC. The cells were cultured in RPMI-1640 medium supplemented (10% fetal bovine serum, 100 U/mL penicillin-streptomycin), and cultured at 37°C in 5% CO2 incubator. The cells were passaged every 3 to 4 days, with 0.25% trypsin (w). The HUVEC cells in the logarithmic growth phase were inoculated in 96-well plate at a density of 1 × 105 cells/mL, 100 µL/well. The cells were incubated at 37°C, 5% CO2 in a cell incubator for 24 h, and the original culture medium was replaced with a fresh medium, and the cells were divided into three steps of the study. The first steps were to explore the effect of incubation duration on changes in HUVECs cell glycocalyx expression. The second steps were to investigate the impact of glucose dose on changes in HUVECs cell glycocalyx expression. The third step was to analyze the comparison of single and intermittent glucose doses on changes in HUVECs cell glycocalyx expression.

Immunofluorescence

Immunofluorescence analysis was carried out according to the procedure in previous studies (Khanmohammadi, Sakai and Taya, 2017). The expression of glycocalyx were detected by the primary antibody of hyaluronic acid (Santa Cruz Biotechnology Inc, Dallas, Texas, USA, Catalog number Sc-221733), heparan sulfate (Antibodies Online, Aachen, Germany, Catalog number ABIN2280658), and syndecan (Bioss Antibodies Inc, Woburn, Massachusetts, USA, Catalog number Bs-1309R-Cy5).

Statistical analysis

Data was presented in mean ± standard deviation. Data were analyzed by ANOVA test using SPSS version 16 for Windows.

Results

Effect of incubation duration on glycocalyx

Figure 1 presents the expression of hyaluronic acid in HUVECs cells. The hyaluronic acid expression tends to increase in 24-hour incubation compared to 12-hour incubation, although it has not been significantly different ($p > 0.05$). Hyaluronic acid expression was significantly lower at 48-hour incubation compared to 12-hour incubation ($p < 0.05$). Hyaluronic acid expression was significantly higher at 72-hour incubation compared to 12 hours, 24 hours, or 48-hour incubation ($p < 0.05$). Hyaluronic acid expression was not significantly different at 48-hour incubation compared to 24-hour incubation ($p > 0.05$).

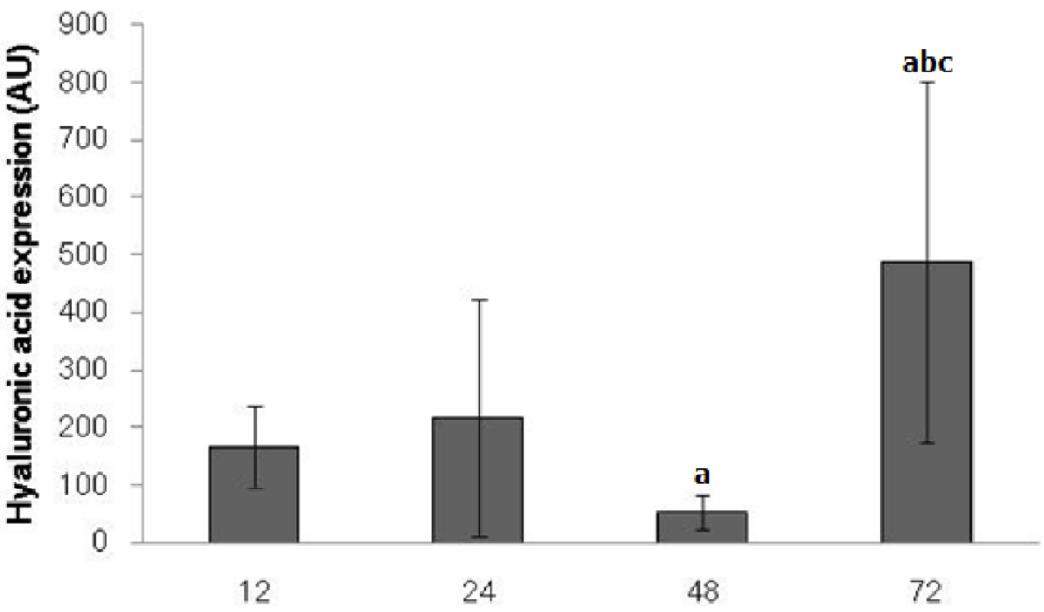

Fig.1. The expression of hyaluronic acid according to incubation time (without glucose exposure). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Heparan sulfate expression in HUVECs cells can be seen in Figure 2. Heparan sulfate expression significantly increased at 24-hour, 48-hour incubation compared to 12-hour incubation ($p < 0.05$). Heparan sulfate expression did not differ significantly at 72-hour incubation compared to 12-hour incubation ($p > 0.05$). Heparan sulfate expression did not differ significantly at 48 hours or 72 hours incubation compared to 24 hours incubation ($p > 0.05$). Heparan sulfate expression did not differ significantly at 72 hours incubation compared to 48 hours incubation ($p > 0.05$).

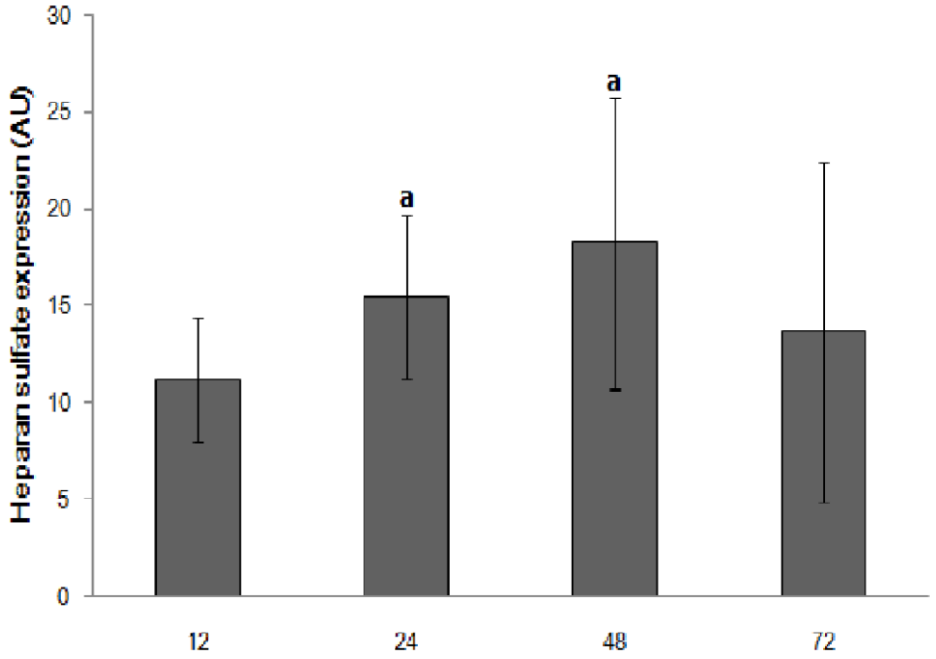

Fig.2:The expression of heparan sulfate according to incubation time (without glucose exposure). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. AU: arbitrary units.

Figure 3 displays the syndecan expression of HUVECs cells in various incubation groups. Syndecan expression was significantly lower at 24-hour, 48-hour, and 72-hour incubation compared to 12-hour incubation ($p < 0.05$). Syndecan expression did not differ significantly at 48 hours or 72 hours incubation compared to 24 hours incubation ($p > 0.05$). Syndecan expression did not differ significantly at 72 hours incubation compared to 48 hours incubation ($p > 0.05$).

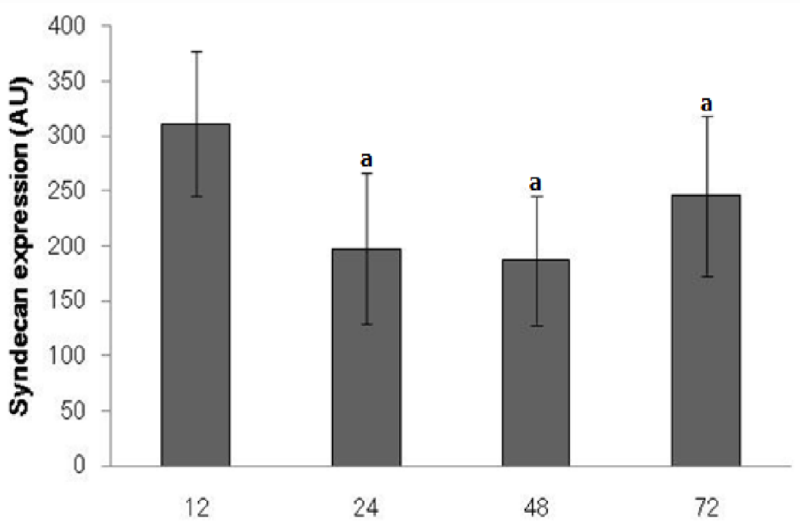

Fig.3:The expression of syndecan according to incubation time (without glucose exposure). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. AU: arbitrary units.

Figure 4 presents the expression of hyaluronic acid in HUVECs exposed to 5 mM glucose. Hyaluronic acid expression was significantly higher at 24-hour, 48-hour, and 72-hour incubation compared to 12-hour incubation ($p < 0.05$). Hyaluronic acid expression was significantly lower at 48-hour incubation than 24-hour incubation ($p < 0.05$). The Hyaluronic acid expression did not differ significantly at 72-hour incubation compared to 24-hour incubation ($p < 0.05$). Hyaluronic acid expression was significantly higher at 72 hours incubation than 48 hours incubation ($p < 0.05$).

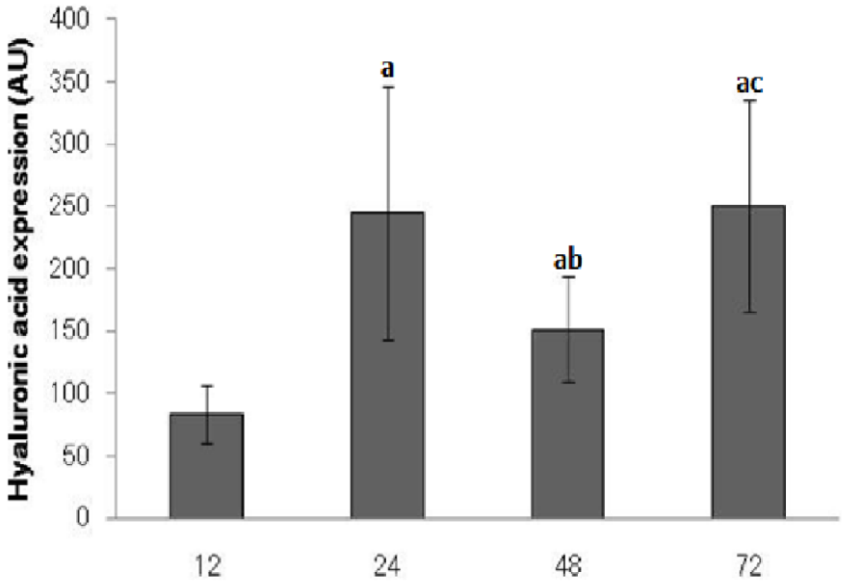

Fig.4. The expression of hyaluronic acid according to incubation time (glucose 5 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Heparan sulfate expression at five mM glucose exposure to HUVECs cells can be seen in Figure 5. Heparan sulfate expression did not differ significantly at 24-hour, 48-hour incubation compared with 12-hour incubation ($p < 0.05$). Heparan sulfate expression significantly decreased at 72-hour incubation compared to 12-hour incubation ($p < 0.05$). Heparan sulfate expression significantly decreased at 48-hour incubation compared to 24-hour incubation ($p > 0.05$). Heparan sulfate expression was significantly lower at 72-hour incubation than 24-hour incubation ($p > 0.05$).

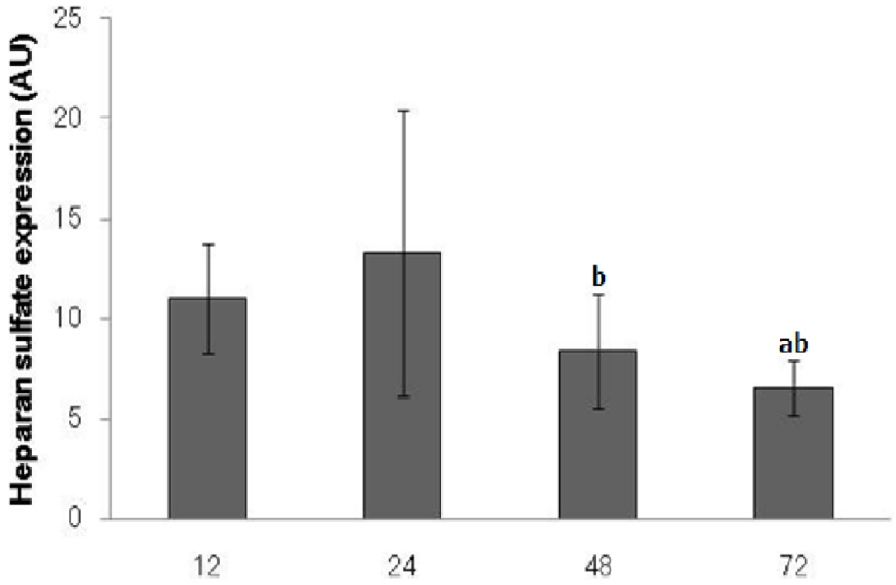

Fig.5. The expression of heparan sulfate according to incubation time (glucose 5 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. AU: arbitrary units.

Figure 6 shows the change in syndecan expression in HUVECs exposed to 5 mM glucose. Syndecan expression significantly increased at 24-hour, 48-hour, and 72-hour incubation compared to 12-hour incubation ($p < 0.05$). Syndecan expression was significantly higher at incubation 48 compared to 24-hour incubation ($p < 0.05$). Syndecan expression did not differ significantly at 72-hour incubation compared to 24-hour incubation ($p > 0.05$). Syndecan expression was significantly lower at 72 hours incubation than 48 hours incubation ($p < 0.05$).

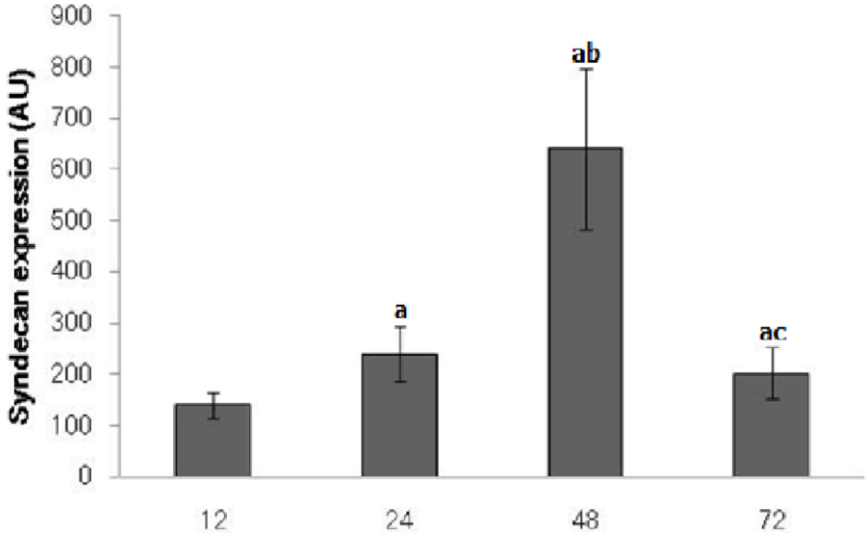

Fig.6:The expression of syndecan according to incubation time (glucose 5 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Hyaluronic acid expression due to high-dose glucose exposure is presented in Figure 7. Hyaluronic acid expression was significantly higher at 24, 48 hours, and 72 hours incubation than 12 hours incubation ($p < 0.05$). Hyaluronic acid expression was not significantly different at 48-hour incubation compared to 24-hour incubation ($p < 0.05$). Hyaluronic acid expression significantly decreased at 72-hour incubation compared to 24-hour incubation ($p > 0.05$). Hyaluronic acid expression significantly decreased at 72 hours incubation compared to 48 hours incubation ($p > 0.05$).

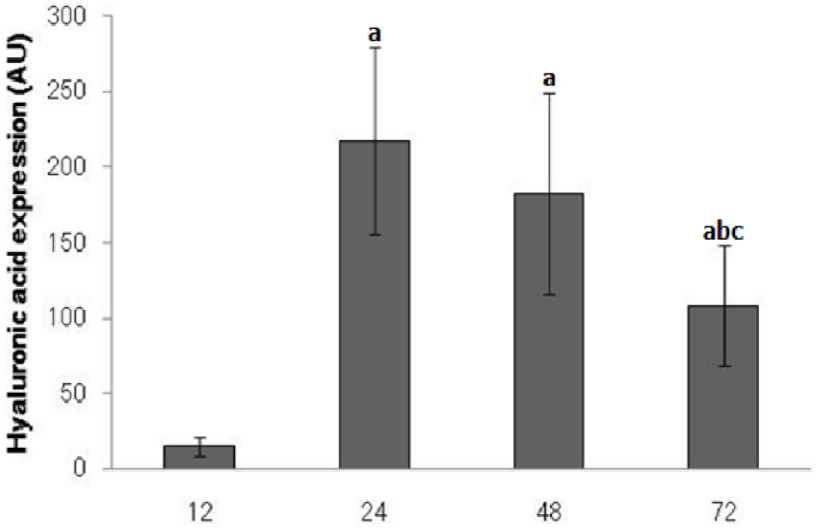

Fig.7:The expression of hyaluronic acid according to incubation time (glucose 22 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Figure 8 displays the expression of heparan sulfate due to high doses of glucose. Heparan sulfate expression did not differ significantly at 24-hour incubation compared with 12-hour incubation ($p > 0.05$). Heparan sulfate expression significantly decreased at 48-hour incubation compared to 12-hour incubation ($p > 0.05$). Heparan sulfate expression did not differ significantly at 72-hour incubation compared to 12-hour incubation ($p > 0.05$). Heparan sulfate expression was significantly lower at 48-hour incubation than 24-hour incubation ($p > 0.05$). Heparan sulfate expression did not differ significantly at 72-hour incubation compared to 24-hour incubation ($p > 0.05$). Heparan sulfate expression was significantly increased in 72-hour incubation compared to 48-hour incubation ($p > 0.05$).

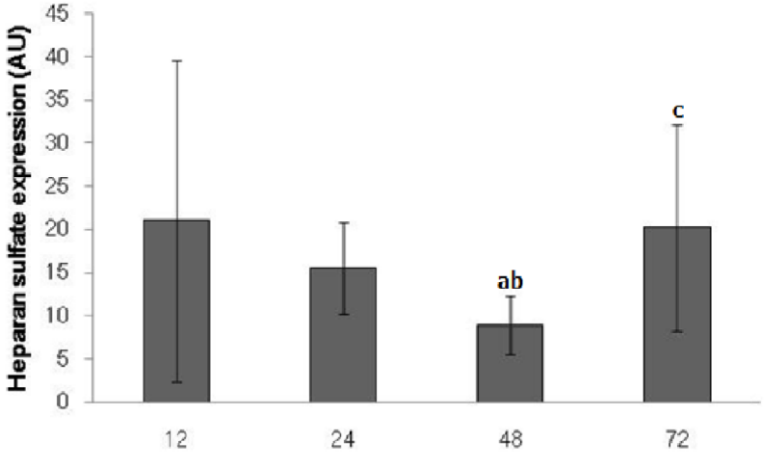

Fig.8:The expression of heparan sulfate according to incubation time (glucose 22 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Syndecan expression due to high-dose glucose exposure can be seen in Figure 9. Syndecan expression was not significantly different at 24-hour incubation than 12-hour incubation ($p < 0.05$). Syndecan expression significantly increased at 48 hours or 72 hours incubation compared to 12 hours incubation ($p < 0.05$). Syndecan expression significantly increased at 48-hour incubation compared to 24-hour incubation ($p < 0.05$). Syndecan expression significantly increased at 72-hour incubation compared to 24-hour or 48-hour incubation ($p < 0.05$).

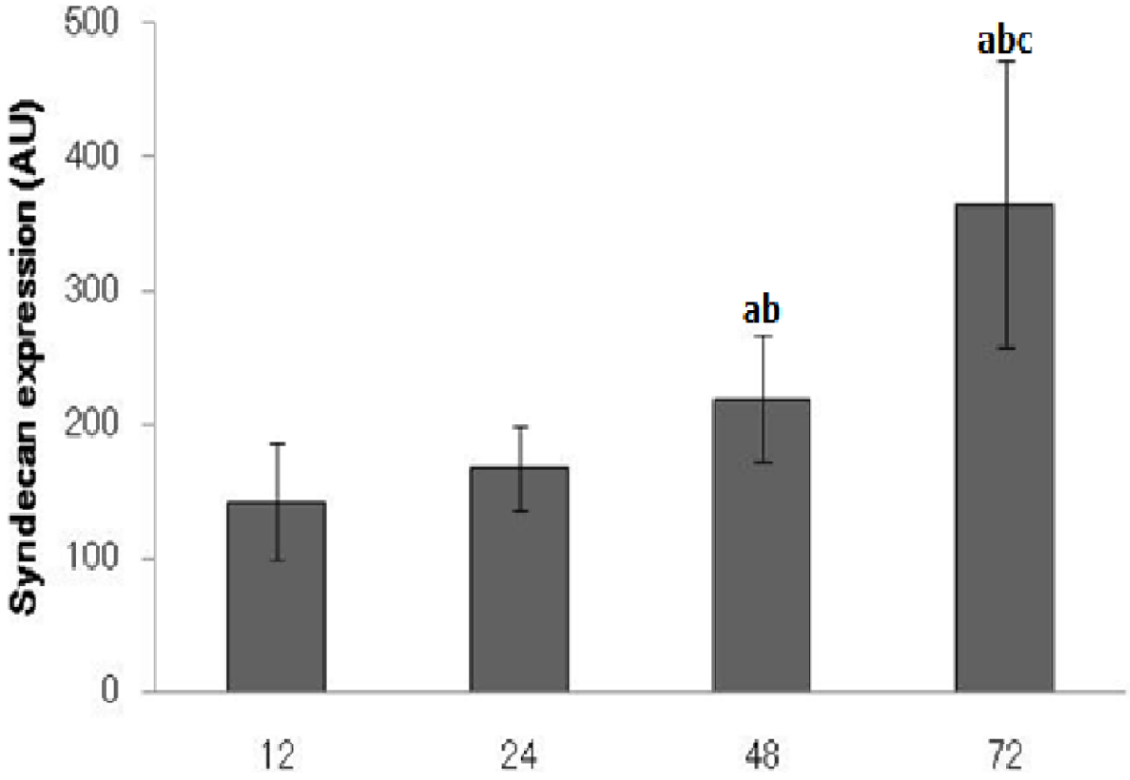

Fig.9:The expression of syndecan according to incubation time (glucose 22 mM). Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with 12-hours of incubation. [b]: p < 0.05 in comparison with 24-hours of incubation. [c]: p < 0.05 in comparison with 48-hours of incubation. AU: arbitrary units.

Effect of glucose dosage on glycocalyx

Figure 10 displays the expression of hyaluronic acid HUVECs cells exposed to various doses of glucose in different incubation periods. In 12-hour incubation, the expression of hyaluronic acid decreased significantly at a dose of 5 mM or 22 mM compared to a glucose dose of 0 mM ($p < 0.05$). Hyaluronic acid expression significantly decreased at 22 mM dose compared to a 5 mM glucose dose ($p < 0.05$). In 24-hour incubation, the expression of hyaluronic acid did not differ significantly at a dose of 5 mM compared to a dose of 0 mM glucose or a dose of 22 mM compared to a dose of 0 mM glucose ($p < 0.05$). At 48 hours incubation, the expression of hyaluronic acid was significantly higher at a dose of 5 mM or 22 mM than a glucose dose of 0 mM ($p < 0.05$). Hyaluronic acid expression was not significantly different at 22 mM compared to 5 mM glucose dose ($p > 0.05$). At 72 hours incubation, the expression of hyaluronic acid did not differ significantly at a dose of 5 mM compared to a glucose dose of 0 mM ($p > 0.05$). Hyaluronic acid expression significantly decreased at a dose of 22 mM compared to a glucose dose of 0 mM ($p < 0.05$). Hyaluronic acid expression significantly decreased at 22 mM dose compared to a 5 mM glucose dose ($p < 0.05$).

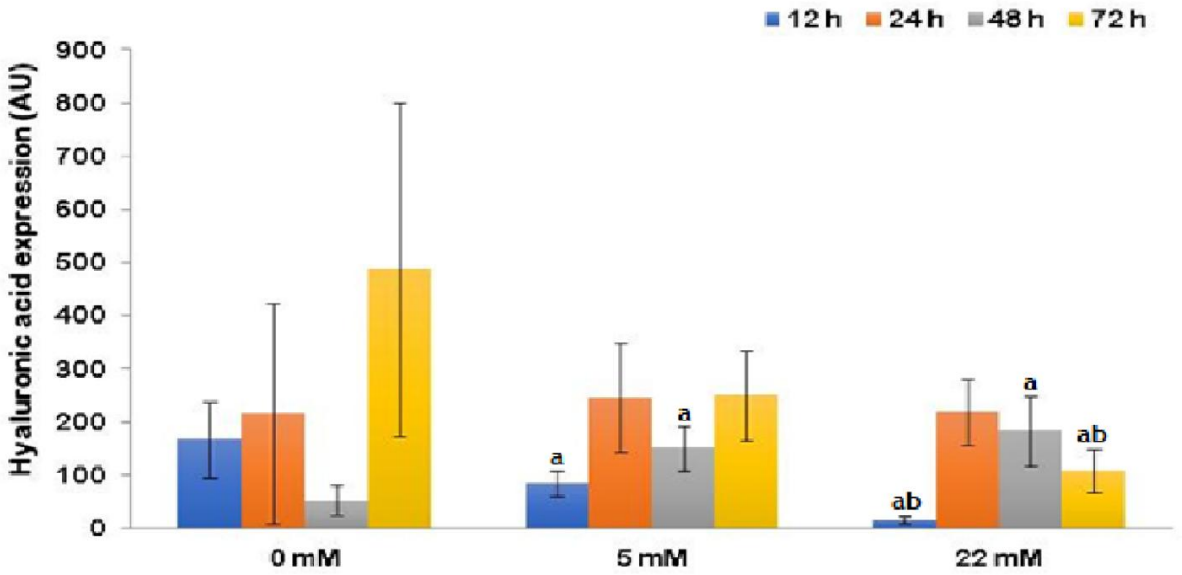

Fig.10:The expression of hyaluronic acid according to glucose level. Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with glucose level at dose of 0 mM. [b]: p < 0.05 in comparison with glucose level at dose of 22 mM. AU: arbitrary units.

Heparan sulfate expression of HUVECs cells exposed to various doses of glucose across multiple incubation periods can be seen in Figure 11. At 12 hours, 24 hours, and 72 hours incubation, heparan sulfate expression did not differ significantly at five mM or 22 mM doses compared to 0 mM or between a dose of 22 mM compared to a dose of 5 mM ($p > 0.05$). At 48 hours incubation, heparan sulfate expression significantly decreased at a dose of 5 mM or 22 mM compared to a glucose dose of 0 mM ($p < 0.05$). Heparan sulfate expression did not differ significantly at a dose of 22 mM compared to a glucose dose of 5 mM ($p > 0.05$).

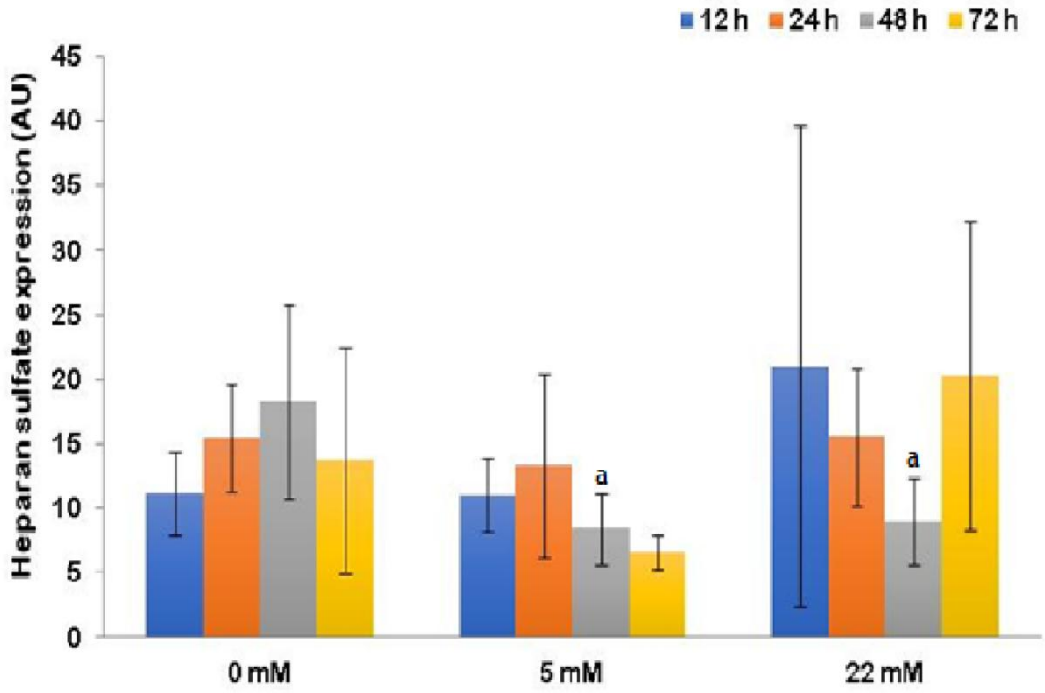

Fig.11:The expression of heparan sulfate according to glucose level. Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with glucose level at dose of 0 mM. AU: arbitrary units.

Figure 12 shows the syndecan expression of HUVECs cells exposed to various doses of glucose in various incubation periods. At 12-hour incubation, syndecan expression was significantly lower at a dose of 5 mM or a dose of 22 mM compared to 0 mM ($p < 0.05$). Syndecan expression did not differ significantly at a dose of 22 mM compared to a dose of 5 mM ($p > 0.05$). At 24-hour incubation, syndecan expression was not significantly different at a dose of 5 mM compared to a dose of 0 mM glucose or a dose of 22 mM compared to a dose of 0 mM glucose ($p < 0.05$). At 48 hours incubation, syndecan expression was significantly higher at five mM dose than glucose dose of 0 mM ($p < 0.05$). Syndecan expression did not differ significantly at a dose of 22 mM compared to a glucose dose of 0 mM ($p < 0.05$). Syndecan expression significantly decreased at 22 mM dose compared to 5 mM glucose dose ($p < 0.05$). At 72 hours incubation, syndecan expression did not differ significantly at a dose of 5 mM compared to a glucose dose of 0 mM ($p > 0.05$). Syndecan expression significantly increased at 22 mM compared to glucose 0 mM ($p < 0.05$). Syndecan expression was significantly higher at 22 mM dose than a five mM glucose dose ($p < 0.05$).

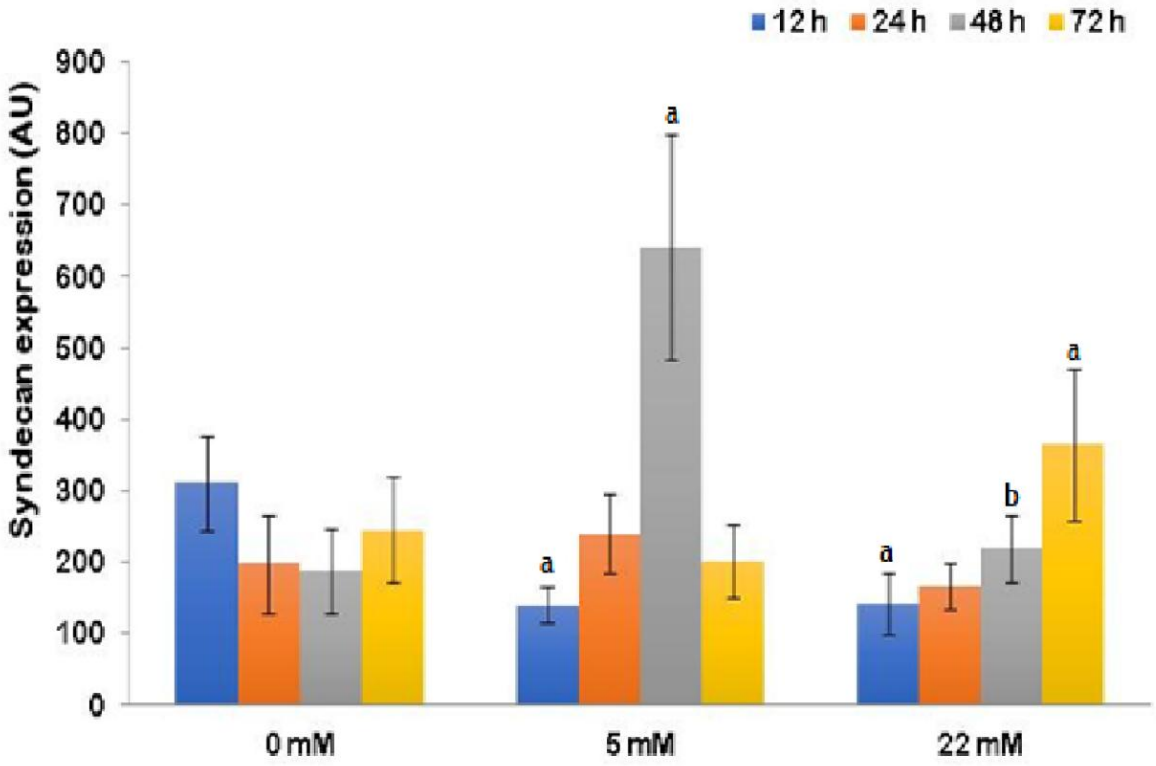

Fig.12:The expression of syndecan according to glucose level. Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with glucose level at dose of 0 mM. [b]: p < 0.05 in comparison with glucose level at dose of 22 mM. AU: arbitrary units.

Comparison of single and intermittent doses

Hyaluronic acid expression in various study groups can be seen in Figure 13. Hyaluronic acid expression was not significantly different in glucose five dose exposure compared with 0 mM glucose dose ($p > 0.05$). Hyaluronic acid expression significantly decreased at exposure to glucose dose 22 compared to glucose dose of 0 mM or intermittent treatment compared to glucose dose of 0 mM ($p < 0.05$). Hyaluronic acid expression was significantly lower in glucose exposure at dose 22 compared with glucose dose of 5 mM ($p > 0.05$). Hyaluronic acid expression was not significantly different in intermittent glucose exposure compared to a 5 mM glucose dose ($p > 0.05$). Hyaluronic acid expression was significantly higher in glucose occasional dose exposure than 22 mM glucose dose ($p > 0.05$).

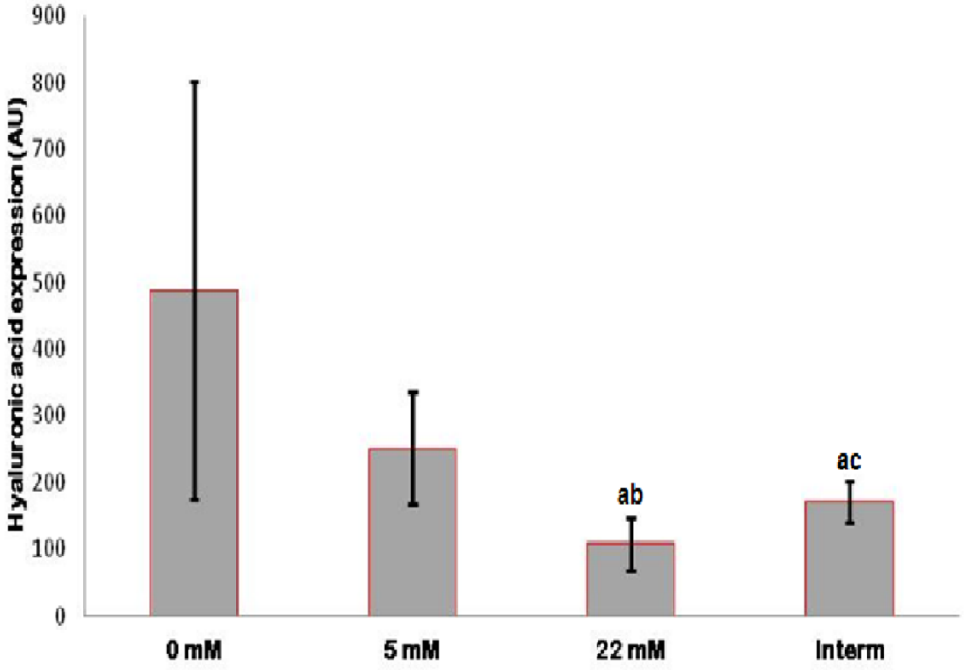

Fig.13:The expression of hyaluronic acid in single and intermittent glucose exposure. Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with glucose level at dose of 0 mM. [b]: p < 0.05 in comparison with glucose level at dose of 5 mM. [c]: p < 0.05 in comparison with glucose level at dose of 22 mM. AU: arbitrary units.

Heparan sulfate expression in various study groups can be seen in Figure 14. Heparan sulfate expression was significantly lower in glucose five dose exposure compared to 0 mM glucose dose ($p < 0.05$). Heparan sulfate expression did not differ significantly in glucose dose 22 compared with glucose dose of 0 mM ($p > 0.05$). Heparan sulfate expression increased significantly in glucose exposure at dose 22 compared to glucose dose of 5 mM ($p < 0.05$). Heparan sulfate expression significantly increased with intermittent glucose exposure compared with five mM or 22 mM glucose ($p < 0.05$).

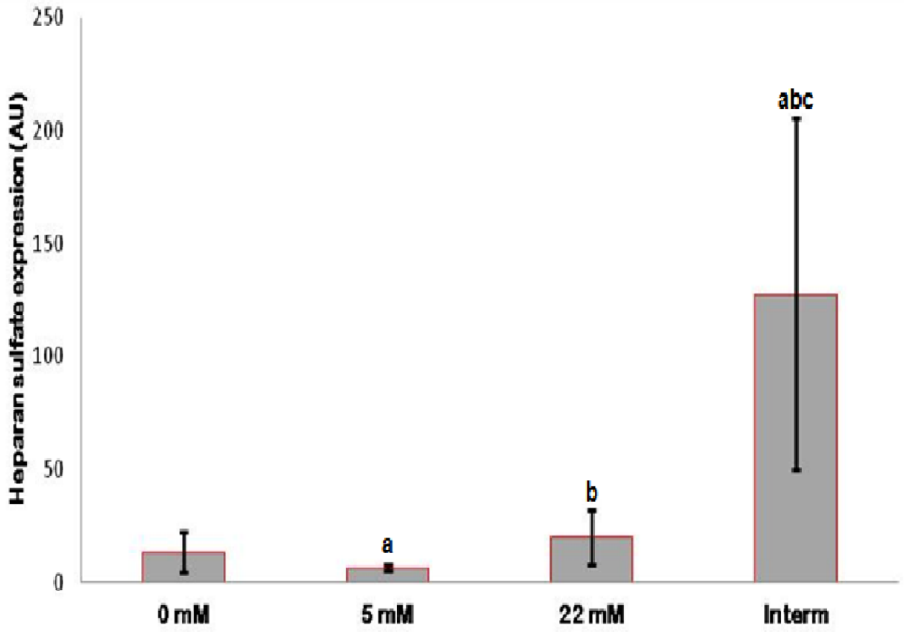

Fig.14:The expression of heparan sulfate in single and intermittent glucose exposure. Note: data was presented as mean ± standart of deviation; [a]: p < 0.05 in comparison with glucose level at dose of 0 mM. [b]: p < 0.05 in comparison with glucose level at dose of 5 mM. [c]: p < 0.05 in comparison with glucose level at dose of 22 mM. AU: arbitrary units.

Syndecan expression in various groups can be seen in Figure 15. Syndecan expression was not significantly different in glucose five dose exposure compared with 0 mM glucose dose ($p > 0.05$). Syndecan expression significantly increased by exposure to glucose 22 doses compared to 0 mM glucose doses ($p < 0.05$). Syndecan expression did not differ significantly in intermittent glucose exposure compared with 0 mM glucose dose ($p > 0.05$). Syndecan expression significantly increased with exposure to glucose 22 doses compared to 5 mM glucose doses ($p < 0.05$). Syndecan expression did not differ significantly in intermittent glucose exposure compared to 5 mM glucose dose ($p > 0.05$). Syndecan expression significantly decreased in intermittent glucose exposure compared to 22 mM glucose dose ($p < 0.05$).

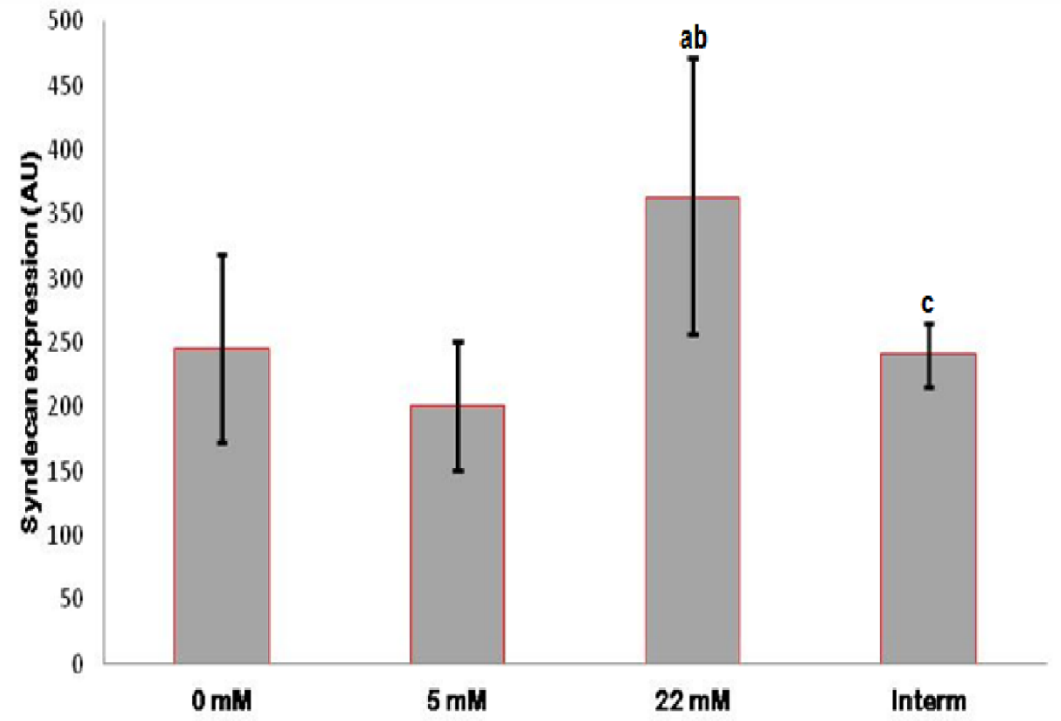

Fig.15:The expression of syndecan in single and intermittent glucose exposure. Note: data was presented as mean ± standart of deviation; [a]: $p < 0.05$ in comparison with glucose level at dose of 0 mM. [b]: $p < 0.05$ in comparison with glucose level at dose of 5 mM. [c]: $p < 0.05$ in comparison with glucose level at dose of 22 mM. AU: arbitrary units.

DISCUSSION

Effect of incubation duration on glycocalyx

In this study, it was proven that hyaluronic acid, heparan sulfate, and syndecan-1 are present on the surface and expressed by HUVECs cells. This finding is consistent with previous findings that glycoprotein and proteoglycans are expressed in HUVECs (Savery et al., 2013; Jing et al., 2016; Li et al., 2016; Khanmohammadi, Sakai and Taya, 2017; Yang et al., 2019).

In this study, the dynamics of hyaluronic acid, heparan sulfate, and syndecan expression were found in HUVECs cells for various incubation periods (without glucose exposure). Hyaluronic acid expression was significantly higher at 72-hour incubation compared to 12-hour, 24-hour, or 48-hour incubation. This indicates that at 72 hours of incubation, there is a tendency toward synthesis compared to the degradation of hyaluronic acid. Hyaluronic acid is synthesized in the plasma membrane through the activation of hyaluronic acid synthase. Hyaluronic acid degradation occurs due to gradual local deterioration (Saegusa, Isaji and Kawarada, 2002; Leskova et al., 2019), or due to hyaluronidase released by endothelial cells (Chajara et al., 2000; Chowdhury et al., 2016). Various enzymes are also involved in the degradation of hyaluronic acid, including thrombin, cathepsin B, proteinase-3, and plasmin (Becker et al., 2015). This study uses M-199 medium containing magnesium. Hyaluronic acid synthase requires magnesium as a catalyst (DeAngelis, 1996).

In this study, the length of incubation did not affect the expression of heparan sulfate. This indicates that changes in the local environment during incubation do not modify the expression of

heparan sulfate exposure. Syndecan was found to be significantly reduced at 24, 48, and 72 hours incubation compared to 12 hours. We suspect that this mechanism of decline is caused by the activation of matrix metalloproteinase (Zeng et al., 2014). Magnesium contained in the M-199 medium will be used for hyaluronic acid synthase catalysts in HUVECs cell membranes. This will result in magnesium deficiency as long as incubation. In HUVECs with magnesium deficiency, there will be an increase in matrix metalloproteinase activity (Ferrè et al., 2010).

On physiological dose glucose exposure (5 mM), there was a significant increase in hyaluronic acid expression at 24-hour, 48-hour, and 72-hour incubation compared to 12-hour incubation. This indicates that glucose exposure at physiological doses is a stimulus to the upregulation of hyaluronic acid HUVECs cells. Hyaluronic acid is glycocalyx, which is synthesized in cell membranes, not in the Golgi apparatus. The synthesis of hyaluronic acid is not only through hyaluronan synthase in cell membranes but is also played by cytoplasmic enzymes that produce uridine-diphosphate (UDP) -glucose precursors (Viola et al., 2008; Gallo et al., 2019). For heparan sulfate, there was a significant decrease in 72-hour incubation compared to 12-hour incubation. The synthesis of heparan sulfate is played by sulfotransferase in the Golgi Apparatus, while degradation of heparan sulfate is played by sulfatase and heparanase (Patel, Pineda and Hoffman, 2017). This shows that exposure to glucose in physiological doses causes degradation of heparan sulfate. We suspect this degradation mechanism occurs through the formation of oxidants from glucose exposure. Monosaccharides undergo autooxidation to form superoxide radicals, hydroxyl radicals, hydrogen peroxide, and carbon-centered radicals (Wolff and Dean, 1987). Glycosaminoglycans can be damaged by the oxidant system (Han and Hiebert, 2013; Marini et al., 2018).

For syndecan, there is a dynamics of fluctuation in syndecan expression on physiological doses of glucose exposure. Syndecan release occurs due to several physiological agents, for example, thrombin, hyperosmolarity, sphingomyelinase, and ceramide (Fitzgerald et al., 2000). Syndecan release due to glucose in physiological levels is thought to be protective against endothelial cells (Wang et al., 2009). Syndecan ectodomains dissolved due to shedding are biologically active and capable of binding to the same ligand (Subramanian, Fitzgerald and Bernfield, 1997).

For high doses of glucose exposure, there was a significant increase in the reduction in hyaluronic acid expression along with an increase in incubation time. This shows that hyaluronic acid undergoes upregulation and then degrades due to exposure to high doses of glucose. At initial incubation, there is dominance in the synthesis of hyaluronic acid through hyaluronan synthase, uridine-diphosphate (UDP)-glucose precursor, and the influence of Mg catalyst (Viola et al., 2008; Gallo et al., 2019). In the final incubation, there is dominance in the direction of degradation by hyaluronidase (Dhulekar and Simionescu, 2018). Previous studies have shown that hyaluronidase can be activated by hyperglycemia, thereby contributing to decreased glycocalyx volume (Dogné et al., 2016). In hyperglycemia, thrombospondin-2 enables HAS-2, thereby increasing plasma levels of hyaluronic acid in diabetic patients (Vigetti et al., 2014). Reactive oxygen compounds are also involved in this process (Eshaq, Wright and Harris, 2014). Reactive oxygen compounds not only have a direct effect on hyaluronic acid but can trigger proteolysis through activation of the metalloproteinase matrix and inactivation of endogenous protease inhibitors. This study extends the previous model that hyaluronic acid degradation occurs in diabetic mice (Wolff and Dean, 1987).

For heparan sulfate, significant degradation was found at 48 hours incubation. Heparan sulfate degradation is played by sulfatase and hepaticase (Patel, Pineda and Hoffman, 2017) as well as pH and mechanical stimulus (Reitsma et al., 2007). For high-dose glucose exposure, there was a significant increase in the expression of syndecan from 48 hours of incubation. This shows the upregulation of syndecan in high glucose levels. We hypothesize that this increased expression is a mechanism of endothelial cell protection against glycocalyx degradation. The glycocalyx function depends on distribution and stability (Zeng et al., 2014). This study is not consistent with previous findings that exposure to high glucose levels can reduce syndecan (Han and Hiebert, 2013).

Effect of glucose dosage on glycocalyx

Under normal conditions, hyaluronic acid is in a massive molecular form (> 500 kDa) and is anti-inflammatory. In inflammatory and disease conditions, hyaluronic acid is degraded into small molecular weight fragments and is proinflammatory (Hull et al., 2015). At 12 and 72 hours of incubation, the higher the dose, the lower the expression of hyaluronic acid in HUVECs cells. This indicates that the 12 and 72 hours incubation, the higher the glucose dose, the greater the degradation of hyaluronic acid. Hyaluronic acid degradation results from gradual local degradation (Saegusa, Isaji and Kawarada,

2002; Leskova et al., 2019), or due to enzymatic activity, including hyaluronidase (Chajara et al., 2000; Chowdhury et al., 2016), thrombin, cathepsin B, proteinase-3, and plasmin (Becker et al., 2015) or due to oxidative stress (Jiang, Liang and Noble, 2011). Hyaluronidase breaks down sizeable molecular weight hyaluronic acid into small molecular weight, but its enzyme activity does not occur in little molecular weight hyaluronic acid (Rügheimer et al., 2009). This study extends previous findings that the degradation of hyaluronic acid in diabetic mice (Leskova et al., 2019), through the activation of hyaluronidase (Dogné et al., 2016), as well as the role of reactive oxygen compounds (Eshaq, Wright and Harris, 2014).

Glycocalyx degradation does not occur in heparan sulfate. Interestingly, syndecan was found to be significantly increased. This shows the upregulation of syndecan in high glucose levels. We hypothesize that this increased expression is a mechanism of endothelial cell protection against glycocalyx degradation. The glycocalyx function depends on distribution and stability (Zeng et al., 2014). This study is not following previous findings that high glucose levels can reduce syndecan expression in endothelial cells (Han and Hiebert, 2013).

Comparison of single and intermittent doses

Previous studies have suggested that fluctuations in glucose concentrations (peak hyperglycemia and hypoglycemia) stimulate the production of reactive oxygen compounds and cellular apoptosis more severe than constant high sugar levels (Risso et al., 2001; Quagliaro et al., 2003; Piconi et al., 2006). If endothelial cells are exposed to stable hyperglycemia, the production of reactive oxygen compounds produced will also be permanent. This will trigger initiation, propagation, and termination reactions. At the time of the termination reaction, every radical produced will always form a neutral compound when the formation of other radicals is stable. When the production of reactive oxygen compounds due to hyperglycemia is fluctuating, the termination reaction is incomplete, and radical compounds are formed and trigger oxidative stress.

In this study, the expression of hyaluronic acid decreased significantly with glucose exposure at dose 22 compared with a dose of glucose 0 mM or intermittent dose compared with glucose at a dose of 0 mM. This reduction is more significant in high-dose exposure than in intermittent doses. This indicates three things: First, high-dose glucose exposure triggers more severe degradation of hyaluronic acid than intermittent glucose exposure. Hyaluronic acid degradation through gradual local degradation (Saegusa, Isaji and Kawarada, 2002; Leskova et al., 2019), hyaluronidase activity (Chajara et al., 2000; Chowdhury et al., 2016; Marini et al., 2018), thrombin, cathepsin B, proteinase-3, and plasmin (Becker et al., 2015), or oxidative stress (Jiang, Liang and Noble, 2011). This finding is not consistent with previous studies that functional injury to HUVECs cells is more severe due to glucose variability than hyperglycemia (Abdelzaher et al., 2016; Guo et al., 2016). Second, the low expression of hyaluronic acid also indicates the ability of endothelial cells to survive oxidative stress. Previous research stated that high molecular hyaluronic acid as an antioxidant (Ye et al., 2012). Scavenging and antioxidant activity are higher in low molecular compared to high molecular hyaluronic acid (Ke et al., 2011). On the other hand, the release of hyaluronic acid from endothelial cells is thought to contribute to the development of complications of diabetes mellitus (Infante et al., 2017).

For heparan sulfate, there is an increase in expression at doses of 22 mM and intermittent doses compared to physiological doses. A more significant improvement was found for intermittent doses. This indicates that exposure to high doses of glucose and intermittent doses triggers the upregulation of heparan sulfate. We suspect that this increase in heparan sulfate is a compensatory mechanism due to the degradation of hyaluronic acid.

For syndecan, there was a significant increase in dose 22 mM compared to doses 5 mM and 0 mM. For intermittent doses, there was no difference compared to treatments 5 and 0. This indicates the upregulation of syndecan in high-dose glucose exposure(Guo et al., 2016). We hypothesize that this increased expression is a mechanism of endothelial cell protection against glycocalyx degradation. The glycocalyx function depends on distribution and stability (Zeng et al., 2014). This study is not by previous findings that high glucose levels can reduce syndecan expression in endothelial cells (Han and Hiebert, 2013; Zhu et al., 2019).

Conclusion

In conclusions, in conditions without glucose exposure and physiological glucose exposure, glycocalyx has a dynamic expression. High glucose exposure eliminates the dynamics of hyaluronic acid and syndecan expression. Single-dose exposure triggers a decrease in hyaluronic acid expression compared to intermittent exposure, and this is the opposite of heparan sulfate exposure.

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Additional information
No additional information is available for this paper.

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