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# Investigating the Endothelial Glycocalyx in Health and Disease in Dogs

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## Introduction

The glycocalyx (GCX) is a gel like matrix covering the luminal surface of endothelial cells. Structurally it is composed of proteins and glycosaminoglycans (GAGs) of which hyaluronan is the only non-sulphated GAG. Functionally the GCX is integral to the maintenance of health, contributing to vascular permeability, intravascular homeostasis and transmission of shear stress forces to endothelial cells[1]. Visualisation of the GCX is challenging, due to its fragility with the most established technique involving vessel perfusion, staining and electron microscopy[2]. The contribution of GCX degradation to multiple disease states is hypothesised, however, as direct GCX visualisation is difficult, degradation can be assessed by quantification of its breakdown products in serum samples. Human studies have identified hyaluronan, a GCX breakdown product, in diseases such as chronic kidney disease and decompensated heart failure[3,4]. The influence of GCX shedding in disease in veterinary medicine is yet to be explored. GCX degradation is plausible is dogs with mitral valve disease (MVD) and is supported by histopathological evidence of endothelial damage and homeostatic dysfunction in these patients. In addition, the importance of GCX in haemostasis also suggests potential damage in hypercoagulable dogs.

## Objectives

**Firstly to perfuse and fix canine arterial samples with Alcian blue to enable pioneering GCX visualisation using electron microscopy. Secondly, to use a commercially available canine ELISA to quantify hyaluronan in dogs with MVD and hypercoagulability and compare to a normal healthy population.**

## Method

Canine uterine and testicular artery samples were perfused with 0.1% Alcian Blue/2.5% glutaraldehyde/0.1M sodium cacodylate and fixed with 2.5% glutaraldehyde/0.1M sodium cacodylate prior to visualisation using transmission electron microscopy. The Study was approved by the University of Bristol Animal Welfare and Ethics Review Board. GCX damage was evaluated by measuring hyaluronan in residual blood samples from dogs with MVD (diagnosed on echocardiography by a board-certified cardiologist), hypercoagulability (G>8 dynes/sec on thromboelastography) and healthy control dogs undergoing blood donation using a commercially available canine ELISA (Quantikine, R&D Systems). Hyaluronan concentrations were compared across groups using the Kruskal-Wallis test. Post-hoc analysis between groups was performed using Mann-Whitney U tests with Bonferroni corrections.

## Results

Glycocalyx was successfully visualised in testicular artery from 1/5 dogs (median [range] glycocalyx depth 68.2 [32.1-122.9] nm) and uterine artery from 1/1 dogs (47.6 [26.1-129.4] nm) (Fig 1). The median (range) hyaluronan concentration in dogs with MVD (n=29), hypercoagulability (n=37) and controls (n=17) was 76.6 (31.1-925.6), 100.7 (16.9-844.4) and 50.1 (18.9-80.22) ng/ul respectively. Dogs with MVD and hypercoagulability had significantly higher hyaluronan concentrations compared to control dogs (P<0.001) (Fig 2).

**Fig 1. Glycocalyx visualised in dog uterine artery**

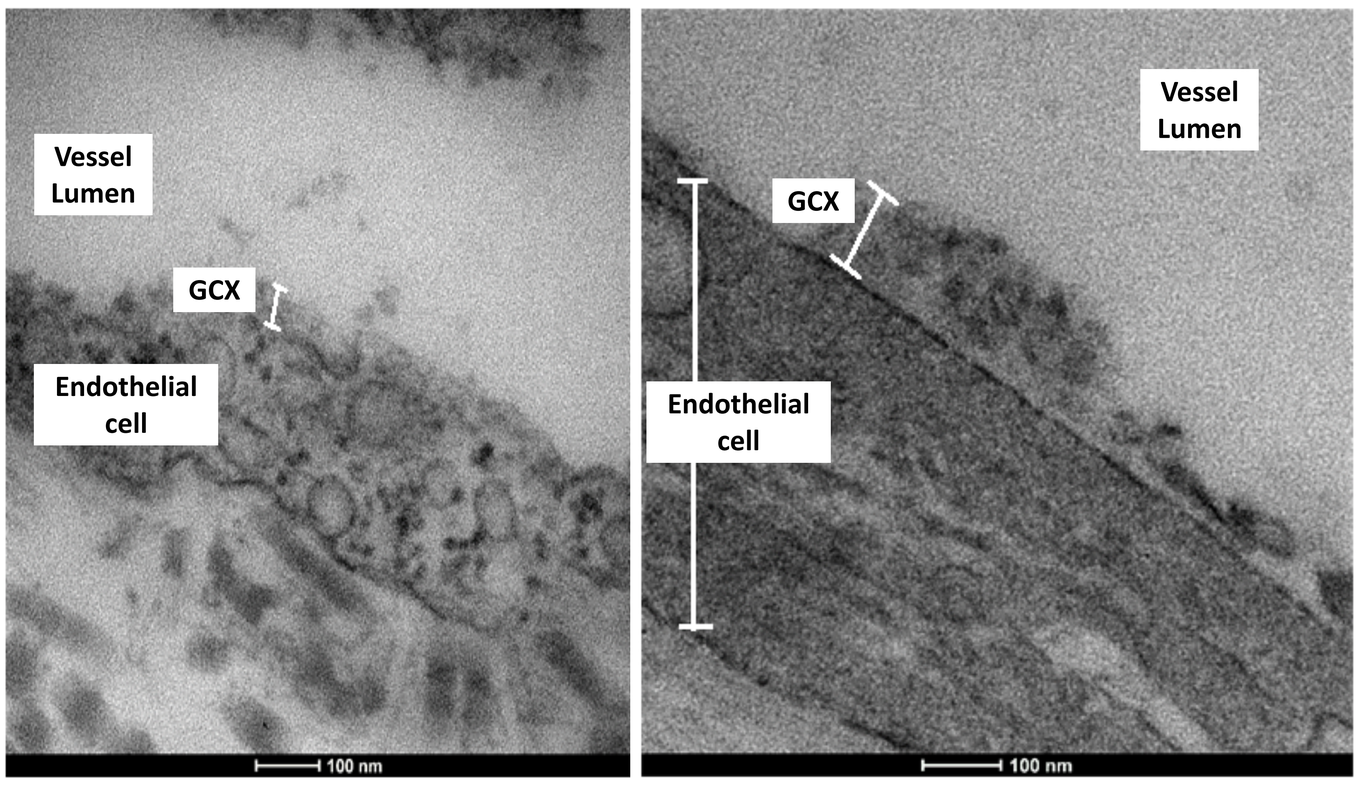


**Figure 1.** Electron micrograph of a dog uterine artery demonstrating the glycocalyx.

**Fig 2. Glycocalyx component, Hyaluronan concentration in clinical populations**

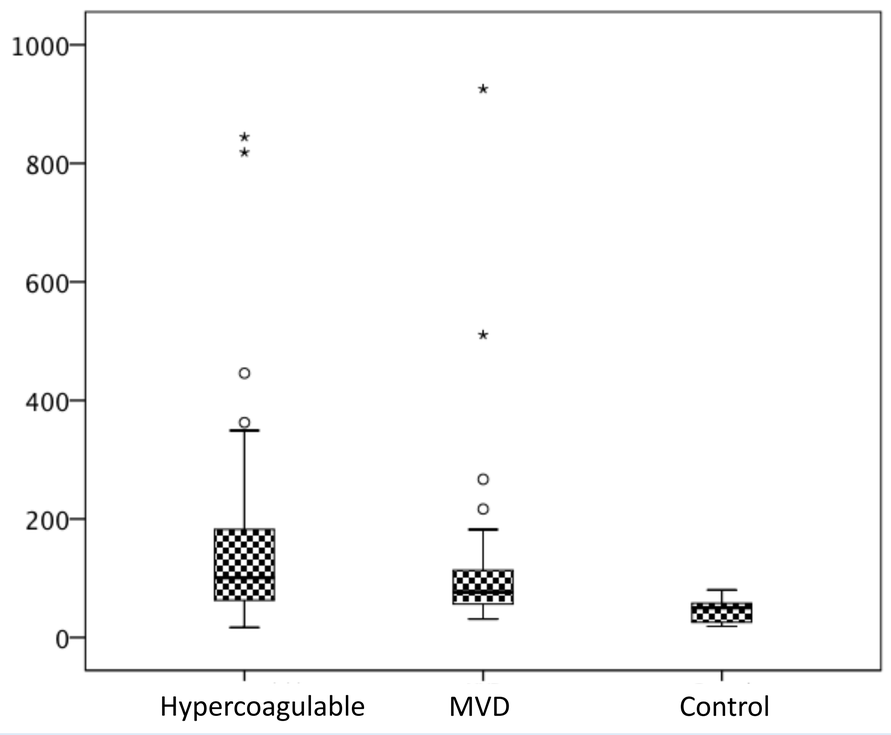


**Figure 2.** Graph to show the median [range] Hyaluronan concentration measured in clinical and normal populations.

## Conclusion

Dogs with MVD and hypercoagulability have increased concentrations of hyaluronan compared to a normal canine population indicating GCX degradation and suggesting it may play a role in the pathogenesis of these diseases. Further studies are required to explore the diagnostic and prognostic value of non-invasive detection of GCX biomarkers. Furthermore, therapies to restore damaged GCX may have the potential to reduce morbidity and mortality in dogs with MVD and hypercoagulability.

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