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Glycated collagen alters endothelial cell actin alignment and nitric oxide release in response to fluid shear stress

Steven F. Kemeny [a], Dannielle S. Figueroa [b], Allison M. Andrews [b], Kenneth A. Barbee [b], Alisa Morss Clyne [a,*]

[a] Drexel University, Department of Mechanical Engineering and Mechanics, USA
[b] Drexel University, School of Biomedical Engineering, Science and Health Systems, USA

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ABSTRACT

People with diabetes suffer from early accelerated atherosclerosis, which contributes to morbidity and mortality from myocardial infarction, stroke, and peripheral vascular disease. Atherosclerosis is thought to initiate at sites of endothelial cell injury. Hyperglycemia, a hallmark of diabetes, leads to non-enzymatic glycosylation (or glycation) of extracellular matrix proteins. Glycated collagen alters endothelial cell function and could be an important factor in atherosclerotic plaque development. This study examined the effect of collagen glycation on endothelial cell response to fluid shear stress. Porcine aortic endothelial cells were grown on native or glycated collagen and exposed to shear stress using an *in vitro* parallel plate system. Cells on native collagen elongated and aligned in the flow direction after 24 h of 20 dynes/cm^2 shear stress, as indicated by a 13% decrease in actin fiber angle distribution standard deviation. However, cells on glycated collagen did not align. Shear stress-mediated nitric oxide release by cells on glycated collagen was half that of cells on native collagen, which correlated with decreased endothelial nitric oxide synthase (eNOS) phosphorylation. Glycated collagen likely inhibited cell shear stress response through altered cell–matrix interactions, since glycated collagen attenuated focal adhesion kinase activation with shear stress. When focal adhesion kinase was pharmacologically blocked in cells on native collagen, eNOS phosphorylation with flow was reduced in a manner similar to that of glycated collagen. These detrimental effects of glycated collagen on endothelial cell response to shear stress may be an important contributor to accelerated atherosclerosis in people with diabetes.

1. Introduction

7.8% of the United States population has diabetes, and another 18.5% are categorized as pre-diabetic (CDC, 2007). Cardiovascular complications are the primary cause of death in diabetic patients (Creager et al., 2003). Diabetes is associated with both macrovascular and microvascular diseases including atherosclerosis, nephropathy, and retinopathy (Grundy et al., 1999). Atherosclerosis, the build-up of fatty plaques at locations of endothelial cell injury, occurs in non-diabetic and diabetic patients alike (Basta et al., 2004; Vlassara and Palace, 2002). However, atherosclerosis develops earlier and is more diffuse in people with diabetes (Vigorita et al., 1980).

Endothelial cells line all blood vessels and maintain cardiovascular homeostasis by controlling permeability, vascular tone, inflammation, and blood vessel growth (Michiels, 2003). Endothelial cells are biochemically and biomechanically supported at their basolateral surface by the basement membrane, a specialized extracellular matrix (ECM). Diabetic hyperglycemia leads to non-enzymatic glycosylation (or glycation) of ECM proteins (Brownlee, 1995; Nakamura et al., 1993). ECM protein glycation leads to endothelial cell dysfunction by altering cell adhesion, spreading, and signaling (Bobbink et al., 1997; Haitoglou et al., 1992; Krishnamurti et al., 1997).

When endothelial cells are exposed to fluid shear stress, they align in the flow direction through focal adhesion and actin fiber reorganization (Dewey et al., 1981; Galbraith et al., 1998; Girard and Nerem, 1995; Goode et al., 1977; Levesque and Nerem, 1985; Malek and Izumo, 1996). Actin fibers align following focal adhesion kinase (FAK) activation at the cell-ECM interface and signaling down the Rho-GTPase pathway (Cary and Guan, 1999; Parsons et al., 2000). Endothelial cells exposed to shear stress also release nitric oxide (NO) (Buga et al., 1991; Corson et al., 1996; Noris et al., 1995). NO is produced through nitric oxide synthase (eNOS) activation, which can occur through the PI3K signaling pathway

* Correspondence to: 3141 Chestnut Street, 170C Alumni Labs, Philadelphia, PA 19104, USA. Tel.: +1 215 895 2366; fax: +1 215 895 1478.
E-mail address: asm67@drexel.edu (A.M. Clyne).

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