Acta Biomaterialia 10 (2014) 1502–1514

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Fibrin-based biomaterials: Modulation of macroscopic properties through rational design at the molecular level ☆

Ashley C. Brown [a,b], Thomas H. Barker [b,c,*]

[a] The School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA
[b] The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA
[c] The Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

ARTICLE INFO

Article history:
Available online 19 September 2013

Keywords:
Fibrin
Biomaterial design
Fibrin glue
Wound repair
Tissue engineering

ABSTRACT

Fibrinogen is one of the primary components of the coagulation cascade and rapidly forms an insoluble matrix following tissue injury. In addition to its important role in hemostasis, fibrin acts as a scaffold for tissue repair and provides important cues for directing cell phenotype following injury. Because of these properties and the ease of polymerization of the material, fibrin has been widely utilized as a biomaterial for over a century. Modifying the macroscopic properties of fibrin, such as elasticity and porosity, has been somewhat elusive until recently, yet with a molecular-level rational design approach it can now be somewhat easily modified through alterations of molecular interactions key to the protein's polymerization process. This review outlines the biochemistry of fibrin and discusses methods for modification of molecular interactions and their application to fibrin based biomaterials.

1. Introduction

Fibrin is one of the classical biomaterials and has been widely utilized for a variety of applications [1–4] since it was first purified in large quantities in the 1940s [5]. Fibrin is at the crux of the coagulation cascade and is formed through the polymerization of the soluble precursor molecule fibrinogen, a process that is initiated by the serine protease thrombin, which is activated in response to injury (reviewed by Weisel and Litvinov [6]). In addition to its important role in hemostasis, following cessation of bleeding, fibrin serves as a scaffold for tissue repair following injury. Furthermore, fibrinopeptides, which are released during polymerization of fibrin, are bioactive themselves and contribute to tissue repair due to their mitogenic, chemotactic and proangiogenic activities [7–9]. Fibrin degradation products are also known activators of wound repair [10–12]. Because of these properties, fibrin serves as a seminal "matrikine", such that the molecule forms an insoluble matrix and also provides chemokines to stimulate surrounding cells.

The fibrin network provides a physical support for neutrophil, macrophage and fibroblast infiltration, which will ultimately lay down fibronectin, collagen and other extracellular matrix (ECM) components to rebuild the damaged tissue. However, like all ECM components, fibrin is not merely a scaffold, but provides a rich wealth of signals and cues to direct cell behaviors following injury through its numerous binding sites for growth factors, integrins and additional ECM components, such as fibronectin, fibulin, thrombospondin [13] and SPARC (secreted protein, acidic and rich in cysteine) [14]. Because of its role in hemostasis and wound repair, fibrin has been used extensively in hemostatic materials, such as fibrin glues, and wound dressings [15–18]. Fibrin has also been utilized for the development of cell instructive scaffolds and is widely utilized for differentiation of stem cells [19,20], stem cell delivery [21] and induction of angiogenesis [22,23].

This wide range of applications of fibrin is due to its rich bioactivity as well as the ease of manipulation of material properties of resulting fibrin gels. Modification of fibrin polymerization dynamics directly affects the porosity, fiber thickness and degree of branching of the polymerized gel, which in turn affects the mechanical properties [24,25]. Fibrin polymerization is a mostly well-understood phenomenon and many groups have exploited the molecular details of this process in order to obtain biomaterials with desired properties for specific applications. Alteration of fibrin polymerization at the molecular level has been achieved through a number of methods, ranging from alterations in pH, salt and thrombin concentration [26,27] to incorporation of polyethylene glycol (PEG) [28] and other polymers [29,30] that can be simply space filling or interact directly with the fibrin molecule [31]. This review will discuss the biochemistry of fibrin structure and polymerization and highlight approaches to exploiting the molecular

☆ Part of the Special Issue on Biological Materials, edited by Professors Thomas H. Barker and Sarah C. Heilshorn.

* Corresponding author at: The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA. Tel.: +1 404 385 5039; fax: +1 404 894 4243.
E-mail address: thomas.barker@bme.gatech.edu (T.H. Barker).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.09.008

mechanisms of fibrin gelation to create biomaterials with a wide range of material properties and applications.

2. Fibrin structure and polymerization

2.1. Biochemistry

Fibrinogen, the circulating inactive precursor of fibrin monomers, is a 340 kDa dimeric glycoprotein, with each dimer being composed of three distinct chains, designated as the Aα, Bβ and γ chains (Fig. 1). Thrombin selectively cleaves fibrinogen to release two sets of peptides 16 and 15 amino acids long, known as fibrinopeptides A and B respectively, from the N termini of the Aα and Bβ chains, resulting in a fibrin molecule now composed of two α, β and γ chains [32]. Each set of α, β and γ chains meet in the central domain of fibrinogen and are linked by disulfide bonds at their N-terminal regions. The α chains are linked by one interchain disulfide bond and the γ chains are linked by two interchain disulfide bonds [33]. The two β chains are not directly linked to one another; a disulfide bond links the β chain of one subunit to the α chain on the other subunit, while the second β chain is linked to the γ chain of the opposite subunit through an additional disulfide bond. This region where the chains are linked is known as the central domain, and is found within the "E" region of fibrinogen. The central domain is flanked on either side by α-helical coiled-coil regions, each stabilized by a pair of disulfide ring structures at their ends, which extend into the distal "D" regions of the molecule. There are two "D" and one "E" region per fibrinogen molecule, with these regions corresponding the proteolytic fragments obtained through complete plasmin degradation [34]. The coiled-coil regions are split approximately equally between the E and D domains. The distal portions of the D regions are composed of the C-termini of the β and γ chains (known as the βC and γC domains, respectively) [35]. The C-termini of the α chains (known as the αC domain) account for approximately two-thirds of the α chains, and are composed of a globular and a linear portion. The αC domains are located near the E region of the molecule and can interact both intramolecularly and intermolecularly [36]. These interactions are discussed subsequently in further detail.

2.2. Polymerization: knob A/knob B

Fibrin polymerization begins when thrombin cleaves fibrinopeptides A and B from the central domain of fibrinogen. This enzymatic cleavage exposes peptide sequences at the N-termini of the α and β chains, termed *knobs A* and *B*, respectively, which are then available to interact with the complementary *holes a* and *b*, located at the C termini of the γ and β chains, respectively (Fig. 2). *Holes a* and *b* do not require enzymatic cleavage to bind to their respective peptides and are always available for interactions. *Knob A* is composed of the N-terminal Gly–Pro–Arg (GPR) motif and is

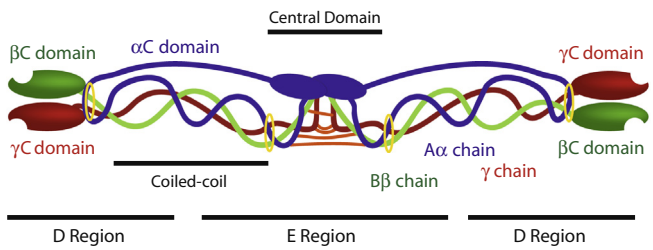

Fig. 1. Fibrinogen structure. Aα chains are shown in blue, Bβ chains are shown in green and γ chains are shown in red. Interchain disulfide bridges connecting the six polypeptide chains in the central domain are shown in orange and disulfide rings stabilizing the coiled-coil regions are shown in yellow.

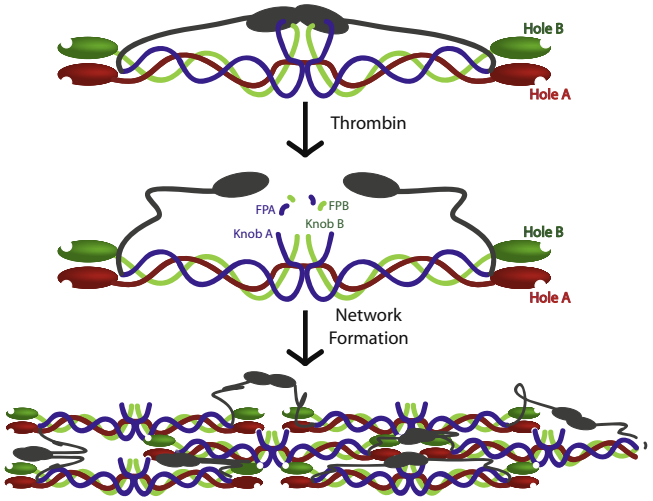

Fig. 2. Fibrin polymerization. α chains are shown in blue, β chains are shown in green and γ chains are shown in red. αC domains are shown in gray.

complementary to *hole a*, located in the γ chains [37]. These *knob A*:*hole a* interactions are high affinity and appear to be the primary contributor to fibrin polymerization [38]. Indeed, fibrin polymerization is inhibited in the presence of high concentrations of a synthetic *knob A* peptide variant of the sequence Gly–Pro–Arg–Pro (GPRP) [39]; for reference, human *knob A* contains the sequence Gly–Pro–Arg–Val (GPRV). The *knob B* motif is composed of the N-terminal Gly–His–Arg–Pro (GHRP) motif and is complementary to *hole b*, located in the β chains. These *knob B*:*hole b* interactions appear to be less crucial than *knob A*:*hole a* interactions in clot formation and fibrin clots can be formed in the absence of *knob B* cleavage [40]. Studies using snake venom enzymes which only cleave *knob A* while leaving *knob B* intact demonstrate that clots formed in the absence of *knob B* interactions displayed thinner fibers than control clots [41]. There is some debate over the physiological relevance and specific functional role of *knob B*:*hole b* interactions; however, these and other studies suggest that *knob B*:*hole b* interactions promote lateral aggregation and play a role in determining clot stability and susceptibility to degradation [42,43].

The energetics of *knob A*:*hole a* and *knob B*:*hole b* interactions vary greatly. In particular, the affinity of *knob B* for fragment D is approximately five times lower than *knob A*, and the strength of *knob B*:*hole b* interactions, as determined through laser tweezing based experiments, is approximately six times lower than that of *knob A*:*hole a* interactions [44,45]. Furthermore, the initial release of fibrinopeptide A (and thus exposure of *knob A*) by thrombin cleavage is significantly faster than the release of fibrinopeptide B and exposure of *knob B* [46] but, as polymerization proceeds, the rate of *knob B* exposure increases, a process which is thought to be driven by conformation changes within fibrinogen. Interestingly, studies utilizing surface bound fibrinogen rather than soluble fibrinogen found that fibrinopeptide B was cleaved faster than fibrinopeptide A [47]. In addition to the classical *knob A*:*hole a* and *knob B*:*hole b* interactions, there is evidence that *knob A*:*hole b* interactions occur while *knob B*:*hole a* interactions do not [38].

Upon initial cleavage by thrombin, *knob A*:*hole a* interactions occur between two adjacent molecules to form non-covalent bonds between the E region of one molecule and the D region of another molecule, resulting in the formation of half-staggered dimer, to which additional molecules are added to give rise to double-stranded protofibrils [48]. It is currently debatable whether *knob B*:*hole b* interactions occur within or between protofibrils [49]; however, recent studies support the hypothesis that *B*:*b* occur