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Review

Soft materials to treat central nervous system injuries: Evaluation of the suitability of non-mammalian fibrin gels

Raivo Uibo [a], Ivo Laidmäe [a,b], Evelyn S. Sawyer [c], Lisa A. Flanagan [d], Penelope C. Georges [e,f], Jessamine P. Winer [e,f], Paul A. Janmey [e,f,*]

[a] Immunology Group, IGMP University of Tartu, Ravila Street 19, Tartu 51014, Estonia
[b] Institute of Pharmacy, University of Tartu, Nooruse Street 1, Tartu 50411, Estonia
[c] Sea Run Holdings Inc., Pine St., Freeport, ME 04032, USA
[d] Department of Pathology, School of Medicine, University of California at Irvine, D440 Medical Sciences I, Irvine, CA 92697-4800, USA
[e] Department of Bioengineering, University of Pennsylvania, 3340 Smith Walk, Philadelphia, PA 19104, USA
[f] Institute for Medicine and Engineering, University of Pennsylvania, 3340 Smith Walk, Philadelphia, PA 19104, USA

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ABSTRACT

Polymeric scaffolds formed from synthetic or natural materials have many applications in tissue engineering and medicine, and multiple material properties need to be optimized for specific applications. Recent studies have emphasized the importance of the scaffolds' mechanical properties to support specific cellular responses in addition to considerations of biochemical interactions, material transport, immunogenicity, and other factors that determine biocompatibility. Fibrin gels formed from purified fibrinogen and thrombin, the final two reactants in the blood coagulation cascade, have long been shown to be effective in wound healing and supporting the growth of cells in vitro and in vivo. Fibrin, even without additional growth factors or other components has potential for use in neuronal wound healing in part because of its mechanical compliance that supports the growth of neurons without activation of glial proliferation. This review summarizes issues related to the use of fibrin gels in neuronal cell contexts, with an emphasis on issues of immunogenicity, and considers the potential advantages and disadvantages of fibrin prepared from non-mammalian sources.

1. Introduction

The elastic properties of tissues and biomaterials designed to promote wound healing or regeneration in specific settings has until recently not been considered as an essential design feature. Most studies have addressed the biochemical and structural properties of scaffolds and extracellular matrices that dictate the molecular specificity of cell adhesions and the transport of soluble factors into and away from the site of repair. A series of recent studies have rejuvenated interest in studying how tissue and biomaterial stiffness influences the structure and function of cells by showing that matrix stiffness, under conditions where other factors are held constant, has a large effect on the rate of cell proliferation, specific programs of gene expression, cell motility, and the developmental fate of stem cells [1–3]. In some cases, matrix stiffness can override chemical stimuli, as illustrated by the lack of response to osteogenic growth factors when mesenchymal stem cells are plated on soft (<1000 Pa) surfaces [4], and in other cases the nature of the adhesive ligand works in concert with substrate mechanics to direct specific processes such as the interplay between the type of integrin ligand and the substrate stiffness on the formation of actin stress fibers or the modulation of motility [5–7].

Not all cells respond similarly to matrix stiffness, and some cell types such as neutrophils seem not to respond to stiffness differences in the range that strongly affect other cell types [5]. One setting in which the elasticity of the substrate appears to have a highly specific effect is in the central nervous system. The brain is among the softest human tissues, with a time-dependent shear storage modulus (or, depending on the type of rheologic measurement, Young's modulus) that varies from > 1000 Pa at millisecond time scales appropriate for modeling effects of impact, to a relatively steady level near 200 Pa at time scales on the order of seconds [8,9]. At sites of injury, where glial scarring occurs, the local stiffness can be palpably higher, but is not yet quantitatively determined, and the stiffness difference at the interface of the glial scar can act as a physical as well as a chemical barrier to neurite extension and neuronal repair in severe injuries [9,10].

The possibility that soft materials might be particularly useful in restoration of diseased CNS tissue is related to the finding that two main cell types of the CNS, neurons and astrocytes, respond in very different ways to matrix stiffness [10], and that gels of low elastic

* Corresponding author. Institute for Medicine and Engineering, University of Pennsylvania, 3340 Smith Walk, Philadelphia, PA 19104, USA.
E-mail address: janmey@mail.med.upenn.edu (P.A. Janmey).

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modulus differentially support the neuronal development of precursor cells [9]. Spinal cord and cortical brain neurons extend neurites and form branches more avidly on soft materials, and are the only cell type thus far documented to be inhibited from extending as the matrix becomes stiffer than the stiffness of a normal brain (<1000 Pa) [9,11–15]. In contrast, astrocytes, like numerous other cell types, develop stress fibers, increase spread area, and become activated on stiff surfaces [10]. This article will focus on evidence of the effects of manipulating substrate stiffness that may have utility in the central nervous system and other injury settings and on the specific properties of matrices derived from non-mammalian clotting factors such as salmon fibrinogen and thrombin that have potential advantages or complementary properties compared to synthetic or human-derived materials.

1.1. Advantages of fibrin from non-mammalian sources

Fibrin has a long and extensive record of use in wound healing including treatment of trauma to the brain and spinal cord [16,17]. Fibrin is the normal scaffold that first forms at sites where trauma to cells initiates the cascade of reactions leading to blood clotting. Purification of the two final reactants, fibrinogen and thrombin, and administration in controlled amounts at defined locations has many clinical applications [18]. The fibrin scaffold can be supplemented with growth factors and other agents for specific settings and is simple to administer, with a straightforward injection into the affected region [19]. The reaction occurs at physiological temperature and pH, and both the rates of gelation and the mechanical properties of the polymerized gel can be controlled easily by adjusting the injection mix [20,21].

Limitations of the use of fibrin in CNS or other injuries include the fact that fibrin is designed to degrade at a rate that depends on the production of plasmin and other proteases generated at the injured site, and in some settings such as neural regeneration, fibrin degradation proceeds too rapidly to allow neurite infiltration without the use of protease inhibitors that can have known or unanticipated negative effects. Cells of the CNS, including neurons, astrocytes, and microglia have receptors for thrombin, fibrinogen or fibrin that also have potentially negative effects on neural wound healing. Human fibrin is also optimized to polymerize at slightly below 37 °C, and fails to clot at lowered temperatures that are required in some surgical settings, including CNS trauma. Additional concerns involve the potential for infectious agents introduced by using proteins derived from pooled human or other mammalian sources. Some of these limitations can be overcome by non-mammalian coagulation proteins, such as have been purified from salmon blood [22–26]. Worldwide production of farmed salmon now exceeds a million metric tons annually. Therefore, millions of liters of blood with consistent quality are available from animals where genetics, nutrition, and environment are controlled or closely monitored.

Proteins derived from salmon or other non-mammalian tissues are likely to be safe from mammalian infectious agents due to the wide evolutionary distance between fish and humans. The low body temperature of cold water fish serves as another barrier to cross-species survival of bacteria or viruses. Prion infection is also most probable in species with similar prion proteins. Although salmon do have a normal isoform of prion protein, its structure is quite different from the mammalian protein [27] and therefore presents little risk. Also, there is no evidence of prion disease in fish, and even if farmed fish ingest mammalian prions, the infectivity is quickly cleared [28]. Prion contamination is a special concern in fibrinogen products, because mammalian fibrinogen can selectively bind the infective part of the prion protein [29].

Salmon fibrinogen and thrombin are sufficiently similar to human fibrinogen and thrombin to be interchangeable in terms of fibrin polymerization, but they differ subtly from those of the human proteins both in amino acid sequence and the nature of glycosylation [23,24,30]. For example, whereas salmon thrombin activates human platelets (a cell type absent in fish) the time course of platelet aggregation in vitro is slightly different, suggesting that salmon thrombin activates the major human platelet receptor, but might not fully activate other cellular targets [23]. The A-alpha chain of salmon fibrinogen has significant lower molecular weight compared to human A-alpha, and the gamma chain of zebrafish fibrinogen, the closest species to salmon that has been sequenced, lacks homology in a region of human fibrinogen that activates microglia [31] and is thought to contribute to inflammation in the CNS.

Differences between salmon and mammalian fibrin are apparent in several studies in which these scaffolds have been compared in cell culture and animal models. Mammalian neurite outgrowth in vitro is significantly less in human or bovine fibrin compared to salmon fibrin in three dimensional fibrin gels [33]. Human fibrinogen inhibits neurite outgrowth while salmon fibrin does not, possibly via outgrowth by triggering an inhibitory signal transduction pathway in neurons [34]. Human fibrinogen polymerizes slowly below 37 °C but salmon fibrinogen clots normally down to 0 °C [30]. Since outcomes can be improved by hypothermia after traumatic brain injury [35] and possibly spinal cord injury [36], salmon fibrin could be effective for the coagulopathy seen at low body temperature.

1.2. Issues of immune response to foreign proteins and biomaterial scaffolds

The same structural differences between salmon and mammalian fibrinogen and thrombin that in some context confers possible advantages to use of non-mammalian fibrin is potentially countered by the presumed higher antigenicity of salmon proteins in a mammalian host. The inflammatory response to synthetic and natural tissue adhesives is variable, depending primarily on the contact of tissue fluids around the biomaterial and the access of host blood cells. In general, host reactions following administration of biomaterials include stages of blood-material interaction, provisional matrix formation, acute inflammation, chronic inflammation, granulation tissue development, foreign body reaction, and fibrosis (fibrous capsule) development [37]. In the cascade of these events the role of the immune system is significant, but immune system activation largely depends on the immunogenicity of tissue adhesives.

Synthetic tissue adhesives, as most widely used and continuously developed, are relatively inert for the host immune system. For example, if administered intravascularly to the rats, N-butyl-2-cyanoacrylate induces only mild eosinophilic inflammation during the first day and after 7 days the tissue reaction is minimal [38]. However, small particles of cyanoacrylates can modulate immune response to external antigens as demonstrated by Simeonova et al. [39]. Currently it is believed that the adjuvant effect of synthetic materials in stimulating dendritic cells and the adaptive immune response to co-expressed (self)antigens may be more important than was previously thought [40]. In addition, systemic inflammatory reactions and septic complications can develop, but the conditions that are needed for their development are not well known. Some of these rare reactions can be life threatening due to fulminant inflammatory reactions [41]. Delayed and recurrent chronic inflammatory and granulomatous reactions could be seen in response to some synthetic gels [42]. However, polyethylene glycol based biodegradable hydrogels used as tissue sealants do not appear to induce immediate humoral or cellular immune reactions [43,44].

Thus in general, synthetic tissue adhesives do not necessarily strongly initiate cross-talk with hosts' tissues and cells due to their relatively passive role in tissue repair. However, the immune reactions largely depend on the chemistry and physics of synthetic biomaterials surfaces which contact the host tissue. Biomaterials' surface characteristics have a significant impact on antigen