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Concentration-dependent rheological properties of ECM hydrogel for intracerebral delivery to a stroke cavity

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ABSTRACT

Biomaterials composed of mammalian extracellular matrix (ECM) promote constructive tissue remodeling with minimal scar tissue formation in many anatomical sites. However, the optimal shape and form of ECM scaffold for each clinical application can vary markedly. ECM hydrogels have been shown to promote chemotaxis and differentiation of neuronal stem cells, but minimally invasive delivery of such scaffold materials to the central nervous system (CNS) would require an injectable form. These ECM materials can be manufactured to exist in fluid phase at room temperature, while forming hydrogels at body temperature in a concentration-dependent fashion. Implantation into the lesion cavity after a stroke could hence provide a means to support endogenous repair mechanisms. Herein, we characterize the rheological properties of an ECM hydrogel composed of urinary bladder matrix (UBM) that influence its delivery and in vivo interaction with host tissue. There was a notable concentration-dependence in viscosity, stiffness, and elasticity; all characteristics important for minimally invasive intracerebral delivery. An efficient MRI-guided injection with drainage of fluid from the cavity is described to assess in situ hydrogel formation and ECM retention at different concentrations (0, 1, 2, 3, 4, and 8 mg/mL). Only ECM concentrations >3 mg/mL gelled within the stroke cavity. Lower concentrations were not retained within the cavity, but extensive permeation of the liquid phase ECM into the peri-infarct area was evident. The concentration of ECM hydrogel is hence an important factor affecting gelation, host-biomaterial interface, as well intra-lesion distribution.

Statement of Significance

Extracellular matrix (ECM) hydrogel promotes constructive tissue remodeling in many tissues. Minimally invasive delivery of such scaffold materials to the central nervous system (CNS) would require an injectable form that exists in fluid phase at room temperature, while forming hydrogels at body temperature in a concentration-dependent fashion. We here report the rheological characterization of an injectable ECM hydrogel and its concentration-dependent delivery into a lesion cavity formed after a stroke based on MRI-guidance. The concentration of ECM determined its retention within the cavity or permeation into tissue and hence influenced its interaction with the host brain. This study demonstrates the importance of understanding the structure-function relationship of biomaterials to guide particular clinical applications.

1. Introduction

Therapeutic options for stroke remain very limited [1]. Most pharmacological agents are administered systemically during the

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acute phase to either resolve a thrombus or to provide neuroprotection. The focus of current therapy is the modulation of the remaining brain tissue response by systemic administration of agents or cells that putatively promote plasticity. In the absence of neuroprotection, cells in the infarct territory die, resulting in liquifactive necrosis and invading phagocytic cells that remove cellular debris and the surrounding tissue matrix [2]. A fluid-filled lesion cavity remains. A key challenge in the treatment of stroke is hence the removal of necrotic debris and access to the adjacent viable or potentially viable tissue.

The advent of regenerative medicine affords potentially novel strategies for integration of endogenous or exogenous cells and/or therapeutic agents/materials into the damaged brain by intracerebral injection [3–5]. Studies have shown that injecting cells directly into the lesion cavity results in their migration into the existing parenchyma with poor survival [6]. These exogenously delivered cells by themselves do not replace lost tissue. To achieve retention of injected cells within the lesion cavity, it is essential to provide a permissive structural and functional microenvironmental niche [6–9]. Such niche support may be achieved by biomaterials specifically engineered to be compatible with neural tissue and amenable to delivery through a small gauge needle for intracerebral injections, with minimal damage to healthy tissue [1,4,10]. Hydrogel forms of naturally occurring biomaterials composed of extracellular matrix (ECM) show in vitro chemoattraction and differentiation stimuli for neural stem cells [11–13]. ECM hydrogels are rapidly infiltrated by pan (CD68+) macrophages, which likely participate in hydrogel degradation [14]. Perhaps most importantly, ECM hydrogels have been shown to promote the M2 "constructive remodeling" macrophage phenotype, characterized as scavenging debris, promoting angiogenesis and recruiting cells involved in constructive tissue remodeling [15,16]. Therefore, ECM hydrogels may supply growth factors, mechanical properties, and/or signaling molecules to support delivered cells; or support surviving endogenous cells and obviate the need for exogenous cells. [5,17–19]. Rheological characterization of an ECM hydrogel intended for CNS applications is essential for an effective evaluation of delivery, safety and efficacy of this therapeutic strategy [20].

The ECM concentration affects rheological properties and determines if it will form a hydrogel or remain in a liquid phase [21]. Without the formation of a gel phase in situ, the ECM will diffuse and not provide a structural support within the lesion cavity [22]. Additionally, the stiffness of hydrogel will influence cell invasion and phenotypic choice of neural progenitors [23,24]. Determining the rheological properties of ECM hydrogels is therefore important to establish the retention of scaffolding material within the lesion cavity and the associated host response. As lesion cavities caused by ischemic stroke may consist of a large volume and irregular shape, it is essential to ensure that administration is indeed into the tissue void rather than intraparenchymally, where this volume would cause tissue disruption and potentially increased intracerebral pressure [25,26]. The use of non-invasive imaging, such as magnetic resonance imaging (MRI), can guide the volume of injection, as well as its stereotactic location, to ensure the safety of this approach [27,28].

The objective of the present study was to characterize the concentration-dependent rheological properties of an ECM hydrogel, specifically an ECM hydrogel composed of urinary bladder matrix (UBM)-ECM, for the intended clinical application of minimally invasive intracerebral injection. To assay the in situ gel formation based on the concentration-dependent properties of the ECM, we also describe an innovative neurosurgical approach for its delivery in the liquid phase into the stroke cavity using MRI guidance.

2. Methods

2.1. Extracellular matrix (ECM)-based hydrogel

The ECM material is composed of the basement membrane and tunica propria of porcine urinary bladder (Tissue Source, Inc., Lafayette, IN). The material was prepared by mechanical delamination of the remaining luminal epithelium and subjacent layers, followed by decellularization by exposure to 0.1% peracetic acid in 4% ethanol (v/v; 120 min; 300 rpm) with agitation followed by a series of PBS and deionized water rinses [21]. Decellularization was confirmed using Hematoxylin & Eosin, 4′,6-diamidino-2-phenylindole (DAPI) staining, agarose gel electrophoresis, and quantification of remnant DNA [29]. The remaining ECM was identified as urinary bladder matrix (UBM), and was then lyophilized, comminuted, solubilized with pepsin (1 mg/mL) in 0.01 N HCl and neutralized with 0.1 N NaOH with dilution to a desired concentration (1, 2, 3, 4, 8 mg/mL) in PBS [21]. A ∅ mg/mL condition consisting of only PBS served as a control.

The final product was an injectable liquid at room temperature (21 °C). Concentration and intracerebral temperature determine the rate of hydrogel formation and the rheological and turbidimetric characteristics of the hydrogel [30,14] which were extensively characterized previously [21]. A concentration >3 mg/mL is required for the formation of hydrogel; below this concentration insufficient gelation is observed. Importantly, if the injectate is excessively diluted within the extracellular fluid (ECF), gelation kinetics and distribution within the lesion can be affected [22,31]. Being able to accurately determine the volume for injection, as well as being able to drain the ECF, is therefore essential to ensure a lesion-specific distribution and robust gelation within the cavity. The rheological properties of hydrogel are important to determine the biophysical interaction with the host tissue.

2.2. ECM hydrogel rheology

All rheological data was collected using a rheometer (AR2000, TA instruments, New Castle, DE) fitted with 40 mm parallel plate geometry, as previously described [21,30,14] and analyzed using the American Society for Testing and Materials (ASTM) standard F2900-11 (Guide for characterization of hydrogels used in regenerative medicine). Temperature was controlled within 0.1 °C using a Peltier plate. At 10 °C, a temperature at which the rate of gelation is negligible, the gel precursor was loaded onto the parallel plate rheometer. Sample evaporation was minimized using mineral oil to seal the edges of the sample-plate interface.

A series of rheological tests were conducted in sequence for each sample. A creep test was performed to measure the steady shear viscosity of the gel precursor by applying a constant shear stress of 1 Pa. An oscillatory time sweep was performed to measure the gelation kinetics of the forming ECM hydrogel by rapidly raising the temperature to 37 °C (a temperature at which ECM gelation occurs) and applying a small 0.5% oscillatory strain at a frequency of 1 rad/s. After 40–60 min when the gelation was deemed complete, an oscillatory frequency sweep was performed to measure the complex viscosity ($|\eta^*|$) over a frequency range (0.1–100 rad/s) by applying 0.5% oscillatory strain. Samples were evaluated in triplicate.

2.3. Middle cerebral artery occlusion (MCAo)

All animal procedures complied with the US Animals Welfare Act (2010) and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). Male Sprague–Dawley rats (260 ± 15 g, Taconic Labs, USA) were

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