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Research Paper

Characterizing the micro-scale elastic modulus of hydrogels for use in regenerative medicine

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ABSTRACT

Our objective was to characterize the elasticity of hydrogel formulations intended to mimic physical properties that cells and tissues experience *in vivo*. Using atomic force microscopy (AFM), we tested a variety of concentrations in a variety of biomaterials, including agarose, alginate, the collagens, fibrin, hyaluronic acid, kerateine, laminin, Matrigel, polyacrylamide, polyethylene glycol diacrylate (PEGDA) and silicone elastomer (polydimethylsiloxane). Manipulations of the concentration of biomaterials were detectable in AFM measurements of elasticity (Young's modulus, E), and E tended to increase with increased concentration. Depending on the biomaterials chosen, and their concentrations, generation of tunable biocompatible hydrogels in the physiologic range is possible.

1. Introduction

Recent work with adult stem/progenitor cells, from sources such as amniotic fluid and placenta, promises to eventually lead to therapies for regeneration of traumatized tissues and organs (De Coppi et al., 2007). However, an effective tissue engineering strategy will require consideration of not only the cells: Another critical component of the tissue engineering strategy is consideration of the stem cell niche, which is the anatomic and functional microenvironment, composed of cells and extracellular matrix, where stem cells reside and self-renew (Lander et al., 2012; Scadden, 2006). Understanding the adult stem/progenitor cell niche will facilitate efforts to manipulate the cells as required experimentally (Kuhn and Tuan, 2010). For example, *in vitro* expansion of rare adult stem/progenitor cells for downstream clinical applications will necessitate that the *in vivo* niche is effectively mimicked (Roeder et al., 2011). The human stem cell niche *in vivo*, whether for embryonic or adult stem cells, is not completely understood (Kiel and Morrison, 2008; Li and Clevers, 2010).

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However, several physicochemical characteristics of the *in vivo* niche have been defined, and these provide a framework for the design of an artificial *in vitro* adult stem/progenitor cell niche. The components of the stem/progenitor cell niche include the structure on which the stem cells are grown, as well as the *in vitro* atmospheric conditions, and the growth media. Specifically, the vital characteristics of these components include: (1) The composition of the scaffold or substrata; (2) The elasticity of the scaffold or substrata; (3) A physiological oxygen tension (Csete, 2005; Estrada et al., 2012); (4) A growth media recipe that is both physiologic and translational (Ludwig et al., 2006). The present study examines components 1 and 2, providing a description of physical properties of various hydrogel recipes.

Atomic force microscopy (AFM), like related micro- and nano-indenter technologies, enables measurement of these physical properties. While alternatives to AFM exist – such as durometers and rheometers – use of AFM for measurement of the elasticity of hydrogels has been widely reported in the literature. Since the time AFM was initially developed (Binnig et al., 1986), it has gained this acceptance as a result of advantages it offers for cell-related measurements. For instance, it has the ability to perform elastic modulus measurements at the micro-scale level, with nanoNewton forces, which is the most relevant scale for cells. Additionally, AFM can be used in liquid or semi-solid environments, relevant to biological experiments (Rehfeldt et al., 2007). Moreover, when using a spherical bead as a probe on a flat substrate, the measurement geometry is simple, and data can be analyzed using well-accepted models. Disadvantages of AFM are that it is relatively slow and tedious to set up and to do measurements. It requires significant expertise to perform these measurements. AFM is well suited to determine the elasticity (Young's modulus) of substrates. It is less well suited to obtain frequency-dependent loss and storage moduli, G' and G'', especially in a liquid environment due to drag forces on the AFM cantilever and probe.

To facilitate clinical translation, there is a need for feeder-cell-free culture systems (Domogatskaya et al., 2008; Unger et al., 2008); as a critical element in the quest for defined conditions, the extracellular matrix (ECM) is of particular interest (Ludwig et al., 2006). The composition of engineered matrices for scaffolds or substrata is determined by the makeup of the corresponding *in vivo* stem/progenitor cell niche. For example, the basal membrane of skeletal muscle cells, under which muscle progenitors (called satellite cells) reside, is composed of collagen type IV, laminin, proteoglycans, and glycoproteins (Ocalan et al., 1988). Thus, the scaffold or substrata for expansion of skeletal muscle progenitor cells is ideally composed of the same materials. The elasticity of the scaffold or substrata should match the elasticity of the corresponding *in vivo* stem/progenitor cell niche. For example, if myogenic differentiation is desired, then the chosen biomaterials should possess an elasticity of ~8–17 kPa, similar to stiffness of normal muscle (Engler et al., 2004, 2006). Embryonic stem (ES) cells, which may adhere to each other as embryoid bodies, have an elasticity of ~0.5 kPa (Chowdhury et al., 2010). Maintenance of stemness in ES cells, and progenitor cell populations, appears to be at least partially matrix-dependent (Dellatore et al., 2008; Discher et al., 2009) because matrix mechanical forces of tensegrity (tensional integrity) (Ainsworth, 2008; Janmey and McCulloch, 2007) guide cell biology and physiology. However, if differentiation of stem/progenitor cells is desired, the stiffness of the scaffold, or hydrogel substrata, can be experimentally manipulated towards this effect. The cells would not necessarily have to reside on the gel directly; they might be seeded on a gel which has been coated appropriately, for example with materials such as collagen, laminin, fibronectin, and so on. Notably, tissue engineering and regenerative medicine are likely to replace 2-D with 2.5-D or 3-D culture methods (Prestwich, 2007). As this happens, biomaterial-based tunable hydrogels onto/into which cells can be seeded would play a key role in either maintaining stemness, or guiding differentiation. Additionally, tissue engineers performing preclinical studies *in vivo* may want control over the material properties of the vehicle into which cells are suspended. For example, if a cells-in-gel format is topically applied to a burn wound (Markert et al., 2010), the gel, when solidified, would ideally exhibit material properties similar to native skin.

In cell culture, the interaction of polymers, such as acrylate-based polymers, with soluble factors in growth media plays a key role in directing stem/progenitor cell fate (Anderson et al., 2004). For example, mesenchymal stem cells (MSCs), grown on collagen-coated polyacrylamide gels engineered to mimic the elasticity of tissues, upregulate markers indicative of differentiation towards cells of those tissues (Engler et al., 2006). The elastic modulus of hydrogels has previously been experimentally manipulated by varying acrylamide and bis-acrylamide concentrations (Engler et al., 2004) or the percentage of polyethylene glycol (PEG) polymer in solution (Gilbert and Blau, 2011; Kloxin et al., 2010a, 2010b). Interactions of cells with materials such as alginate (Lee and Mooney, 2012), collagen (Grinnell, 2003), hyaluronic acid (Shu et al., 2002), polyacrylamide (Engler et al., 2006), and polydimethylsiloxane (PDMS) (Tan et al., 2003) have all been extensively characterized. However, although cell-gel interactions have been characterized, the material properties of the gels themselves have not been evaluated in a head-to-head comparison. Appreciating these observations, we sought to define the elasticity of various biomaterial-based hydrogels, while providing easily reproducible hydrogel recipes, and while using a single AFM methodology for measurement of the hydrogels' material properties. These data would provide much-needed opportunities for hypothesis testing of the correlation between matrix elasticity and maintenance of an undifferentiated state in progenitor cells (Holst et al., 2010), or the correlation between matrix elasticity and differentiation fate (Engler et al., 2006), with biomaterials that are potentially clinically translational.

Our experiments seek to define the elasticity of biomaterials that would serve as thick coatings for culture vessels, or as components of scaffolds or substrata, or as vehicles for topical administration of cell therapies, further allowing experimental control over a bioengineered stem/progenitor cell niche. We used biomaterials which are currently used in *applied* studies and are readily available. Methodical studies of the *basic* mechanisms involved in cell-ECM interactions, with "pure" biomaterials such as polyacrylamide (PA), have been previously reported; *e.g.* (Engler et al., 2006; Rehfeldt et al., 2007). Importantly, as described below, we investigated