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Research Paper

The mechanics of hyaluronic acid/adipic acid dihydrazide hydrogel: Towards developing a vessel for delivery of preadipocytes to native tissues

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ABSTRACT

Promising treatment approaches in repairing tissue defects include implementation of regenerative medicine strategies, particularly delivery of preadipocytes to sites where adipose tissue damage needs to be repaired or where fat needs to be generated. In this study, we suggest that the injectable hyaluronic acid/adipic acid dihydrazide (HA/ADH) hydrogel may be an adipose-tissue-like material in terms of biological compatibility as well as mechanical behavior. First, we show that the hydrogel enables and supports growth, proliferation and differentiation of 3T3-L1 preadipocytes. Second, given that adipose tissue is a weight-bearing biological structure, we investigate the large deformation mechanical behavior of the hydrogel with and without embedded preadipocytes, by performing confined and unconfined compression tests and then calibrating a strain energy density (SED) function to the results. Four test groups were examined: (1) Hydrogel specimens right after the preparation without cells, (2) and (3) 3-days-cultured hydrogel specimens with and without cells, respectively, and (4) 6-days-cultured hydrogel specimens with cells. A one-term Ogden SED was found to adequately describe the hyperelastic behavior of the hydrogel specimens in all experimental groups. Importantly, we found that the mechanical properties of the hydrogel, when subjected to compression, are in good agreement with those of native adipose tissue, with the better fit occurring 3–6 days after preparation of the hydrogel. Third, computational finite element studies of the mechanical (stress–strain)

Abbreviations: ADSC, Adipose-derived stem cells; ATCC, American type culture collection; DDW, Double distilled water; DM, Differentiation medium; DMEM, Dulbecco's modified eagle medium; ECM, Extracellular matrix; FBS, Fetal bovine serum; FE, Finite element; GM, Growth medium; HA/ADH, Hyaluronic acid/adipic acid dihydrazide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LD, Lipid droplet; Oxi-HA, Oxidated hyaluronic acid; PBS, Phosphate-buffered saline; Pen–Strep, Penicillin–streptomycin; SED, Strain energy density; VOI, Volume of interest.

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behavior of the HA/ADH hydrogel when containing mature adipocytes indicated that the stiffnesses of the constructs were mildly affected by the presence of the adipocytes. Hence, we conclude that injectable HA/ADH hydrogel may serve as a vessel for protecting preadipocytes during, and at a short-term after delivery to native tissues, e.g. in research towards regenerative medicine in tissue reconstructions.

1. Introduction

More than 1-million reconstructive and correctional surgical operations are performed every year worldwide in order to repair subdermal defects caused by trauma (burns, wounds etc.), tumor resections or congenital abnormalities (Kim et al., 2012; Patel et al., 2005). These contour defects not only affect patients cosmetically, but also impact their emotional well-being and may even impair functional abilities, such as range of motion. Tissue transplantations (i.e. use of autografts, allografts or xenografts) may be useful to correct contour deficiencies, however these procedures are limited due to lack of donors, donor site morbidity or antigenicity. Hence, promising treatment approaches in repairing tissue defects include implementation of regenerative medicine strategies, particularly delivery of preadipocytes to sites where adipose tissue damage needs to be repaired or where fat needs to be generated (Kundu and Kundu, 2012; Patel et al., 2005).

Hydrogels, which are able to absorb a significant amount of water or biological fluids (Yu and Ding, 2008) have been recognized as having a variety of advantages in the fields of tissue engineering, including their degradation time and their mechanical properties which are similar to the extracellular matrix (ECM) of different soft tissues (Drury and Mooney, 2003; Tan et al., 2009). More specifically, in situ forming hydrogel scaffolds allow the repair of irregular defects without wrinkling, and lessen the risk of implant migration due to strong tissue-hydrogel interfaces that hold the implant in place. Another benefit of in situ forming hydrogels is homogeneous incorporation of cells and bioactive molecules or drugs, as well as easy delivery to the target site (Kundu and Kundu, 2012; Su et al., 2010). Thermosensitive hydrogels are especially attractive due to their spontaneous gelation under the body temperature, which eliminates the need for a chemical reaction or external heating for achieving polymerization (Yu and Ding, 2008).

Many researchers have focused their work on developing injectable hydrogels for different applications, for example, for cartilage or bone tissue repair (Burdick and Anseth, 2002; Elisseeff et al., 1999; Fujimoto et al., 2009; Ziane et al., 2012), and specifically, for developing tissue-engineered fat (Halberstadt et al., 2002; Kim et al., 2012, Ogushi et al., 2012; Song et al., 2010; Tan et al., 2009, 2010; Young et al., 2011). Tan et al. (2009, 2010), for instance, developed animated hyaluronic acid-g-poly (*N*-isopropylacrylamide) hydrogels and multiarm poly(ethylene glycol)-based hydrogels, which were found to promote survival of human adipose-derived stem cells (ADSC). Song et al. (2010) developed a hydrogel composed of chitosan, glycerophosphate and collagen, which again, showed good cell viability outcomes when encapsulated with human ADSC. In an attempt to better mimic the ECM of native adipose tissue, Young et al. (2011) generated an injectable adipose matrix scaffold by removing both the cellular and lipid contents of human lipoaspirate, and their hydrogel was found to support growth and survival of patient-matched ADSC in vitro. However, the production of their hydrogel required a surgical procedure or the availability of donors. More recently, Kim et al. (2012) demonstrated that ADSC encapsulated in alginate hydrogels are a suitable means of delivery of the cells to the target site for regenerative medicine in adipose reconstructions. In a related study, Ogushi et al. (2012) suggested carboxymethylcellulose-based hydrogel as the vehicle of delivery of ADSC. Lastly, Halberstadt et al. (2002) introduced hydrogels based on alginate, which was covalently linked with fibronectin—a cell adhesion protein. When preadipocytes were seeded in these materials and then the cell-gel construct was injected into the subcutaneous space of a sheep, new adipose tissue was formed within the transplant site. Nevertheless, none of these studies (Halberstadt et al., 2002; Kim et al., 2012; Ogushi et al., 2012; Song et al., 2010; Tan et al., 2009, 2010; Young et al., 2011) characterized the mechanical properties of the proposed engineered constructs. Adipose tissue is a weight-bearing biological structure which undergoes large deformations during supported postures—e.g. peaks of nearly 50% compressive strain during sitting as evident by weight-bearing MRI (Linder-Ganz et al., 2007). Hence, the mechanical properties of the constructs cannot be ignored when developing regenerative medicine-based treatments.

In this study, we used the injectable hyaluronic acid/adipic acid dihydrazide (HA/ADH) hydrogel, which was introduced previously as an optically-transparent substitute for nucleus pulposus regeneration or for vitreous humour after vitrectomy (Su et al., 2010, 2011). In these previous studies, only nucleus pulposus cells were successfully cultured in this hydrogel type (Su et al., 2010). We found that the HA/ADH hydrogel is particularly amenable for regenerative medicine applications in adipose tissues considering: (1) The short gelation time at the body's temperature (<3 min), which hence shortens the waiting time for patients in terms of clinical applications, and also prevents extrusion of the hydrogel. (2) The transparency of the hydrogel, which allows straight-forward optical observations in vitro without chemically (e.g. fixation, staining) or otherwise interfering the cells. (3) The hydrogel can be injected through a small gauge needle (17G), thereby minimizing harmful effects that might evolve in surrounding tissues. (4) A simple filtered-sterilization method is sufficient to prepare the hydrogel for culturing. The other injectable hydrogels that were previously reported to be suitable for tissue-engineered fat (as described above) had substantially greater gelation times, ranging between 12 min and over 4 h (Tan et al., 2009, 2010; Song et al., 2010), were not transparent (Halberstadt et al., 2002; Kim et al., 2012,