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Characterization and assessment of hyperelastic and elastic properties of decellularized human adipose tissues

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ABSTRACT

Decellularized adipose tissue (DAT) has shown potential as a regenerative scaffold for plastic and reconstructive surgery to augment or replace damaged or missing adipose tissue (e.g. following lumpectomy or mastectomy). The mechanical properties of soft tissue substitutes are of paramount importance in restoring the natural shape and appearance of the affected tissues, and mechanical mismatching can lead to unpredictable scar tissue formation and poor implant integration. The goal of this work was to assess the linear elastic and hyperelastic properties of decellularized human adipose tissue and compare them to those of normal breast adipose tissue. To assess the influence of the adipose depot source on the mechanical properties of the resultant decellularized scaffolds, we performed indentation tests on DAT samples sourced from adipose tissue isolated from the breast, subcutaneous abdominal region, omentum, pericardial depot and thymic remnant, and their corresponding force–displacement data were acquired. Elastic and hyperelastic parameters were estimated using inverse finite element algorithms. Subsequently, a simulation was conducted in which the estimated hyperelastic parameters were tested in a real human breast model under gravity loading in order to assess the suitability of the scaffolds for implantation. Results of these tests showed that in the human breast, the DAT would show similar deformability to that of native normal tissue. Using the measured hyperelastic parameters, we were able to assess whether DAT derived from different depots exhibited different intrinsic nonlinearities. Results showed that DAT sourced from varying regions of the body exhibited little intrinsic nonlinearity, with no statistically significant differences between the groups.

1. Introduction

A tissue-engineered adipose substitute is valuable to plastic surgeons in a broad range of reconstructive and cosmetic procedures that require the replacement or addition of adipose tissue (Katz et al., 1999, Patrick, 2000, Gimble et al. (2013). A promising strategy to enable soft tissue augmentation involves designing scaffolds that act as tissue substitutes to maintain the desired 3-D volume and guide regeneration of patients' own healthy tissues (Beahm et al., 2003). To date, various types of synthetic and naturally derived scaffolds have been investigated for this application. Such scaffolds have been designed to support cellular attachment and adipogenesis, allowing integration into the host (Flynn and Woodhouse, 2008). Matching the mechanical properties of native tissues is a critical design parameter (Greenwald and Berry, 2000), as too rigid scaffolds cause mechanical irritation and scar tissue formation in the surrounding regions, whereas too soft biomaterials are prone to structural collapse causing implant failure (Patrick et al., 2002).

Among the various types of engineered scaffolds, decellularized adipose tissue (DAT)-based biomaterials derived from extracellular matrix (ECM) of fat have shown particular promise based on their natural ability to support fat formation (Flynn, 2010, Turner et al., 2012, Wu et al., 2012, Young and Christman, 2012, Yu et al., 2013). Adipose tissue represents an abundant source of human ECM that is available from surgeries, such as breast reduction or abdominoplasty. To engineer an off-the-shelf bioscaffold for allogenic applications, the adipose tissue must be processed through chemical,

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biological and/or physical means in order to remove cells and lipid from the tissue while preserving the ECM, ensuring long-term stability and reducing the possibility of immunogenic reactions (Badylak, 2002, Flynn, 2010). These decellularization processes extract the lipid-filled mature adipocytes that comprise the bulk of tissue, potentially altering the normal adipose tissue mechanical properties. Moreover, some decellularization methods may cause changes in the structure and composition of native ECM that further influence the mechanical linear and nonlinear characteristics. At the macroscopic level, the mechanical properties of soft tissues can be primarily characterized by linear elastic parameters, while hyperelastic parameters carry tissue microstructure information (Fung, 1993).

The main objective of this work is to demonstrate that DAT material derived from various adipose depots in the body possess mechanical characteristics necessary for 1) adipocyte infiltration and proliferation, and 2) having similar large deformation characteristics under physiological loading after implantation. In this article, linear elastic and hyperelastic parameters of human DAT samples were measured in order to characterize their properties in the context of developing tissue substitutes for reconstructive breast surgery. Because the composition and structure of the ECM can vary significantly depending on the specific tissue source (Badylak, 2002), our study included a comparative assessment of DAT sourced from multiple depots including the breast, subcutaneous (SC) abdominal region, omentum, pericardial depot, and thymic remnant. While linear elastic parameters are essential to provide stiffness and deformability information at a macroscopic (tissue) level, hyperelastic parameters carry information of tissue microstructure and its changes under finite deformation. Indentation techniques developed by Samani et al. (2003) and Samani and Plewes (2007) were utilized to measure the linear elastic and hyperelastic parameters of DAT produced using a standardized decellularization protocol described in Section 2 (Flynn, 2010). Various strain energy functions were used in the tissue FE models, and their corresponding hyperelasticity parameters were determined. The properties of breast adipose tissue were characterized in a previous study (Samani et al., 2007) and were referenced as a benchmark for the ideal properties of DAT-based biomaterials for breast reconstruction. Finally, to evaluate the deformation of a breast with DAT implant, a breast composed of DAT with the estimated hyperelastic parameters was simulated using FE method, and the resulting deformation was compared to deformation expected to occur with the breast consisting of natural breast adipose tissue.

2. Methods

2.1. Adipose tissue decellularization and ECM characterization

Samples were prepared from adipose tissue collected from breast (4-donors), SC abdominal region (2-donors), omentum (2-donors), pericardial depot (2-donors) and thymic remnant (1-donor) with at least four samples from each donor during surgery at the Kingston General Hospital or Hotel Dieu Hospital in Kingston, Canada, with Research Ethics Board approval (REB # CHEM-002-07) from Queen's University. The samples were transported to the Flynn laboratory at Queen's University in sterile phosphate-buffered saline (PBS) on ice within 1 h of extraction, and sectioned into samples 20–25 g in mass before being subjected to an established 5-day detergent-free decellularization process (Flynn, 2010). In brief, the protocol involved a combination of mechanical, chemical and enzymatic treatment, designed to extract cells and lipids, while preserving structural components of the ECM. Decellularization was assessed histologically by Masson's trichrome staining, as well as DAPI staining to confirm absence of nuclei. This was followed by scanning electron microscopy (SEM) scanning to confirm effective extraction of cells and cell debris, and also assess the ultrastructure of the ECM. Details of these methods are provided in Section A3 of the Appendix.

2.2. Indentation testing

Indentation testing was performed to measure the elastic and hyperelastic parameters of the DAT samples. The indentation was conducted using the apparatus described in Kaster et al. (2011). As illustrated in Fig. 1, the apparatus consists of a load cell along with a linear servo-actuator and computer controller. The actuator was equipped with a circular, plane-ended indenter with 1.5 mm diameter. The amplitude of indentation was chosen to be 0.5 mm in order to achieve an appropriate amount of strain. The indentation was performed with sinusoidal cycles of 0.1 Hz frequency to maintain quasi-static loading.

2.3. Measurement protocol and indentation data acquisition

The tissue specimens were kept in a sterile PBS and refrigerated until the indentation test was performed, at which point they were warmed to room temperature. Each specimen was indented at its center after a preload of 0.1 g was applied to ensure indenter and tissue full contact. After preloading, 20 sinusoidal preconditioning indentation cycles were performed, followed by a minimum number of five cycles with force–displacement data acquisition. Once the force–displacement data were acquired, the smoothest cycle of each data set was chosen for linear elastic and hyperelastic parameter calculation using inverse FE techniques described later.

2.4. Finite element mesh generation

The model of tested DAT samples were cylindrical with 10 mm diameter. The samples height was variable, with an average value of ~3 mm. To generate the FE mesh of each cylinder shaped-model using hexahedral elements, transfinite interpolation (TFI) technique was applied (Knupp and Steinberg, 1994), leading to the axisymmetric mesh shown in Fig. 2. The figure indicates that the mesh size is consistent with expected stress concentration underneath and in the vicinity of the indented area. FE mesh density was determined based on mesh convergence analysis, which involved incrementally refining the FE mesh until the output reached convergence (O'Hagan and Samani, 2008).

2.5. Young's modulus calculation

To calculate the Young's Modulus (E) of each specimen, only the initial linear portion of the force–displacement curve was taken into account. The inverse FE method described in Samani et al. (2003) was utilized, which is based on the following equation:

$$E = \kappa S \tag{1}$$

where S is the slope of the force–displacement data, and κ is a conversion factor determined based on the geometry of the specimen and indenter, and boundary conditions. κ is determined using the specimen's indentation FE model where an arbitrary $E(E_{arb})$ is input, leading to output force–displacement slope (S_{out}) followed by calculating $\kappa = E_{arb}/S_{out}$ as described in Samani et al. (2003). After calculation, statistical analysis using methods described in the Appendix were conducted on the calculated E data to assess their differences statistical significance.

2.6. Hyperelastic parameters

Four strain energy models commonly used with soft tissues were employed in this work to characterize the DAT hyperelastic properties. These models are described in the supplementary Appendix. We used an optimization algorithm to determine the hyperelastic parameters of tissue specimens. O'Hagan and Samani (2008) developed the algorithm, which works iteratively starting with an initial guess of the parameters. In each iteration, the set of parameters is updated systematically to minimize the sum of squared differences between the force–displacement data calculated by sample's FE model and its measured counterpart. For parameter updation, the slope variation algorithm of O'Hagan and Samani (2008) was used for the Yeoh and polynomial models, while the Simplex method

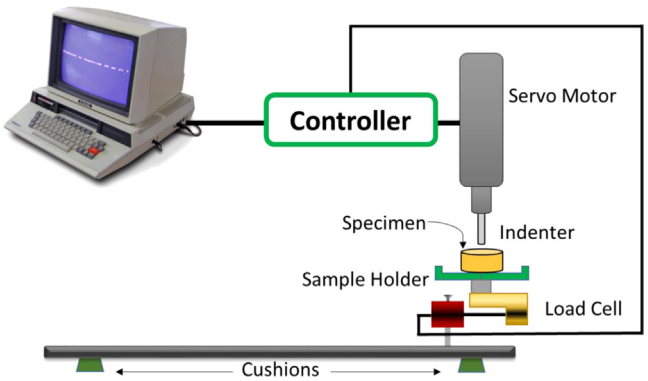

Fig. 1. Indentation device employed to acquire the tissue mechanical response.