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A mathematical model for the determination of forming tissue moduli in needled-nonwoven scaffolds

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ABSTRACT

Formation of engineering tissues (ET) remains an important scientific area of investigation for both clinical translational and mechanobiological studies. Needled-nonwoven (NNW) scaffolds represent one of the most ubiquitous biomaterials based on their well-documented capacity to sustain tissue formation and the unique property of substantial construct stiffness amplification, the latter allowing for very sensitive determination of forming tissue modulus. Yet, their use in more fundamental studies is hampered by the lack of: (1) substantial understanding of the mechanics of the NNW scaffold itself under finite deformations and means to model the complex mechanical interactions between scaffold fibers, cells, and de novo tissue; and (2) rational models with reliable predictive capabilities describing their evolving mechanical properties and their response to mechanical stimulation. Our objective is to quantify the mechanical properties of the forming ET phase in constructs that utilize NNW scaffolds. We present herein a novel mathematical model to quantify their stiffness based on explicit considerations of the modulation of NNW scaffold fiber-fiber interactions and effective fiber stiffness by surrounding de novo ECM. Specifically, fibers in NNW scaffolds are effectively stiffer than if acting alone due to extensive fiber-fiber cross-over points that impart changes in fiber geometry, particularly crimp wavelength and amplitude. Fiber-fiber interactions in NNW scaffolds also play significant role in the bulk anisotropy of the material, mainly due to fiber buckling and large translational out-of-plane displacements occurring to fibers undergoing contraction. To calibrate the model parameters, we mechanically tested impregnated NNW scaffolds with polyacrylamide (PAM) gels with a wide range of moduli with values chosen to mimic the effects of surrounding tissues on the scaffold fiber network. Results indicated a high degree of model fidelity over a wide range of planar strains. Lastly, we illustrated the impact of our modeling approach quantifying the stiffness of engineered ECM after in vitro incubation and early stages of in vivo implantation obtained in a concurrent study of engineered tissue pulmonary valves in an ovine model.

Statement of Significance

Regenerative medicine has the potential to fully restore diseased tissues or entire organs with engineered tissues. Needled-nonwoven scaffolds can be employed to serve as the support for their growth. However, there is a lack of understanding of the mechanics of these materials and their interactions with the forming tissues. We developed a mathematical model for these scaffold-tissue composites to quantify the mechanical properties of the forming tissues. Firstly, these measurements are pivotal to achieve functional requirements for tissue engineering implants; however, the theoretical development yielded critical insight into particular mechanisms and behaviors of these scaffolds that were not possible to conjecture without the insight given by modeling, let alone describe or foresee a priori.

1. Introduction

Regenerative medicine has led to new therapies being used to treat a number of pathologies (mainly wound healing and orthopedics) and has potential to significantly impact medicine as whole by providing the ability to fully restore diseased and injured tissues or entire organs [1]. Such approaches require the development of engineered tissues (ET), and more recently there is growing interest in utilizing ET technologies to perform pharmacological screening [2] or to systematically investigate cellular

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mechanobiology and interactions between cells and extracellular matrix (ECM) [3–5]. Scaffold materials play important roles as they can mimic the native ECM environment [6], modulate cell behavior [7], and dictate structure and function of the ET [8]. Scaffold technologies that support these approaches remain an important scientific area of investigation and development.

Historically, a diverse number of different scaffold technologies have been explored and utilized in a multitude of ET applications. Decellularized native tissues offer excellent structural similarities to the goal tissue structures [9], however host body reactions and long-term functionality due to strong detergents and decellularization agents raise concerns [10,11]. Degradable synthetic scaffolds, such as those made from polymeric materials, represent some of the earliest materials used to support the growth of ET [12]. Materials such as polyglycolic acid (PGA) and polylactic acid (PLA) are attractive due to their well-defined chemical, biological, and mechanical properties. These materials have seen frequent use as they easily degrade in vivo, can be tailored for diverse applications, and have general acceptance within the medical community.

Multi-filament needled-nonwovens (NNW) scaffolds made of bioresorbable PGA and/or PLA crimped fibers represent one the earliest and most ubiquitous biomaterials used as ET scaffolds [13]. During manufacture, fibers are crimped and a needle-array punch entangles fibers sufficiently to bind them (although there is no direct adhesion between fibers), and yields an interconnected porous structure highly suitable for scaffold-based ET approaches [14,15]. NNW scaffolds are sufficiently strong and mechanically stable, although fiber slippage results in limited elastic recovery under tensile loading [16,17]. They continue to serve an important role in ET formation, both as a benchmark for evaluating novel biomaterials and for their unique capacity to promote tissue formation, the latter arising from their intricate microstructure and micromechanics. They are attractive in that the bulk stiffness of the scaffold-tissue construct is exquisitely sensitive to changes in the forming ECM modulus. This is particularly useful when studying the early stages of tissue formation where the magnitude of the ECM moduli are typical rather small (<10 kPa).

From a biomechanics perspective, a fundamental issue is the lack of information concerning the mechanics of the NNW scaffold itself and the complex mechanical interactions between nonwoven scaffolds, cells, and especially the de novo forming ECM. Thus, while NNW scaffolds have yielded promising results in diverse ET applications such as heart valves [18,19], bladder [20], blood vessels [21], and cartilage [22], their use in more fundamental studies is hampered by the lack of: (1) substantial understanding of the complex interactions in between degrading scaffold and forming de novo tissues; and (2) rational models with reliable predictive capabilities describing the evolving mechanical properties of cell-seeded ET constructs based on NNW scaffolds and their response to mechanical stimulation. Engineered tissue approaches not only are well positioned for the study of cellular mechanobiology as they serve as controllable and reproducible testbed for hypothesis formulation and verification, but also present themselves as highly appealing alternative to synthetic implants once and if the functional requirements of clinical application are met. Indeed, both aspects of the ET approach are in fact needed and work synergistically together.

Engelmayr and Sacks proposed the first microstructural model to describe and predict the mechanical response of NNW scaffolds in their virgin state [13]. The model consisted of an extension to the model originally proposed by Freeston and Platt [23] to account for undulated fibers that bend (as opposed to straight fibers that extend) and for the co-existence of fiber-fiber needling points that physically bond fibers and fiber-fiber entanglements. Fiber-fiber cross-over points are responsible for changing the effective fiber crimp (and not the intrinsic crimp imparted to fibers during fabrication), specifically in effective crimp amplitude, wavelength, and consequently, their mechanical response [24]. In another study with cell-seeded NNW scaffolds incubated under cyclic flexure, Engelmayr et al. observed a strong positive linear relation between construct effective stiffness and engineered ECM deposition [25]. The traditional rule-of-mixtures was not able to describe the effective stiffness of the scaffold-ECM composite (substantially underestimated the experimentally measured stiffness), and demonstrated that additional reinforcement mechanisms must be acting within the scaffold-ECM composite. The authors proposed that the principal reinforcement mechanism acting in scaffold-ECM composited is an increase in the number of rigidly-bound fiber-fiber cross-over points, with a concomitant decrease in effective arc length and amplitude of the curved segments spanning in between these points, and thus resulting in effectively stiffer fibers. Their study demonstrated that the extension of curved fiber segments in between fiber-fiber cross-over points is a fundamental mechanism occurring during the deformation of NNW scaffolds, and most importantly, the impact of surrounding ECM in the modulation of the effective scaffold stiffness. However, their study was limited to flexure as the only mode of deformation and strains achieved were of the order of magnitude <5%. More robust models are necessary to understand and frame the complex mechanical behavior of NNW scaffolds, and richer deformations regimes are needed to motivate and drive their development, specifically the behavior of NNW scaffolds and ET constructs based on those under multi-axial strain.

Our group has been interested in developing an improved fundamental understanding of the underpinnings of ET growth and development through highly-integrative approaches bridging modeling-simulation-experimentation to address critical barriers in the field of tissue engineering and its over-reliance on pure empiricism [26,27]. For example, we have developed a modeling framework for engineered tissue formation in NNW scaffolds under mechanical stimulation. Mechanical training is widely recognized as one of the most relevant methods to enhance ET accretion and microstructure, leading to improved mechanical behavior and function; however, the understanding of the underlying mechanisms remains rather limited. In order to explain such augmentation of production and stiffness observed by Engelmayr et al. [25] in dynamically flexed NNW scaffolds, we have hypothesized that mechanical deformation of the porous scaffolds introduces a pumping mechanism that relieves diffusional constraints and supplies more nutrients to cells and consequently enhance their synthetic behavior [26]. However, the improved biochemical environment was not sufficient to explain the substantial improvement observed in another scaffold system (electrospun PEUU [27]) that is able to undergo large deformations (up to 50% stretch), thus reinforcing the role of mechanical deformation in the direct stimulation of the synthetic behavior of cells [27]. An improved understanding of the process of mechanical stimuli transfer, i.e. from the scaffold to the cells, in these mechano-sensitive cell-scaffold systems will lead to more rational design and manufacturing of ETs operating under highly demanding mechanical environments.

In the present study, we extend our previous flexure-based studies on NNW scaffolds to develop a robust mathematical model to predict the mechanical response of the forming ECM by the stiffness amplification of NNW scaffolds under more generalized planar loading states. We illustrate the impact of our modeling approach by employing it to quantify the stiffness of engineered ECM after in vitro incubation and early stages of in vivo implantation obtained in a concurrent study of engineered tissue pulmonary valves in an ovine model [28,29].