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Acta Biomaterialia 4 (2008) 808–816
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A mechanical evaluation of three decellularization methods in the design of a xenogeneic scaffold for tissue engineering the temporomandibular joint disc

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Received 22 August 2007; received in revised form 11 January 2008; accepted 15 January 2008
Available online 7 February 2008

Abstract

Tissue-engineered temporomandibular joint (TMJ) discs offer a viable treatment option for patients with severe joint internal derangement. To date, only a handful of TMJ tissue engineering studies have been carried out and all have incorporated the use of synthetic scaffold materials. These current scaffolds have shown limited success in recapitulating morphological and functional aspects of the native disc tissue. The present study is the first to investigate the potential of a xenogeneic scaffold for use in tissue engineering the TMJ disc. The effects of decellularization agents on the disc's mechanical properties were assessed using three common decellularization protocols: Triton X-100, sodium dodecyl sulfate (SDS) and an acetone/ethanol solution. Decellularized scaffolds were subsequently characterized through cyclic mechanical testing at physiologically relevant frequencies to determine which chemical agent most accurately preserved the native tissue properties. Results have shown that porcine discs treated with SDS most closely matched the energy dissipation capabilities and resistance to deformation of the native tissue. Treatments using Triton X-100 caused the resultant tissue to become relatively softer with inferior energy dissipation capabilities, while treatment using acetone/ethanol led to a significantly stiffer and dehydrated material. These findings support the potential of a porcine-derived scaffold decellularized by SDS as a xenograft for TMJ disc reconstruction.

Keywords: Tissue engineering; Temporomandibular joint; Xenogeneic scaffold; Mechanical properties; Hysteresis

1. Introduction

The temporomandibular joint is a diarthroidal joint that joins the mandible, or lower jaw, to the temporal bone of the skull. One of the most frequently used joints in the body, the TMJ is also often considered the most complex joint [1]. The vast complexity of the TMJ yields itself to a wide variety of pathologies, collectively termed temporomandibular disorders (TMDs). Epidemiological studies have reported that as many as 20–25% of the population experience symptoms of TMDs, which can range from painless clicking and locking of the jaw to debilitating pain and dysfunction [2]. Of these conditions, ~70% of patients seeking treatment for TMDs suffer from a form of internal derangement in which the fibrocartilaginous disc exhibits an abnormal relationship to the condyle, fossa and articular eminence [3]. However, despite the large number of TMJ related conditions, the TMJ remains one of the least studied joints in the body.

Current treatment methods for TMDs are widely varied and are generally regarded as experimental among surgeons due to marginal success rates [4,5]. For example, alloplastic disc replacements such as Proplast–Teflon and Silastic implants were popular in the 1970s and 1980s.

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doi:10.1016/j.actbio.2008.01.016

However, by the 1990s the literature was replete with reports of detrimental effects of these implants. Namely, the implants were prone to fragmentation and tearing, which led to complications such as bone resorption and osteoarthritis. Although the FDA issued an advisory to surgeons to discontinue their use, many patients still suffer from complications caused by the implants [6].

In light of this, the field of tissue engineering offers a unique alternative to treating cases of internal derangement and other complications when the TMJ disc is damaged beyond repair. Tissue engineering of the TMJ disc is still in its infancy, however, with as few as 10 comprehensive studies present in the literature. One of the issues raised at the world's first TMJ Bioengineering Conference in 2006 was that of a need to develop a suitable scaffold material for engineering the disc [6]. Suggested bioscaffolds have included photopolymers [7] and alginate hydrogels [8], but successfully duplicating the complex morphology of the TMJ disc as well as its widely variant mechanical properties may require other alternatives such as xenogeneic extracellular matrix scaffolds. Unlike scaffolds comprised of synthetic materials, xenogeneic scaffolds are inherently suited to facilitate cell adhesion and native remodeling processes. Promising results with tissue xenografts have been reported in several animal and clinical studies. For example, scaffolds derived from porcine urinary bladder submucosa and small intestinal submucosa have been used as xenografts to reconstruct musculoskeletal structures, cardiovascular tissues and skin [9–11].

It is known that the method of decellularization, which is an important step to reduce the immune impact of allogenic/xenogeneic scaffolds, can dramatically alter the mechanical properties of the resulting scaffold [11]. As such, it is the aim of these investigations to demonstrate the potential of a porcine TMJ disc xenograft as a scaffold for engineering disc replacements. Accordingly, we have examined the effects of three decellularization methods on the biomechanical integrity and energy dissipating capabilities of the derived scaffolds. Two common surfactant-based decellularization protocols were chosen for decellularization (sodium dodecyl sulfate (SDS) and Triton X-100) as well as an alcohol-based solution comprising 25% acetone and 75% ethanol. Such alcohol-based solutions have been used previously, albeit in combination with other chemical treatments [12]. Comparative analysis between the resultant acellular scaffolds has been investigated to assess the feasibility of using one of these decellularization methods to create a TMJ disc substitute.

2. Materials and methods

2.1. Specimen preparation

Fresh TMJ discs were collected from the jaws of pigs ages 6–9 months through Animal Technologies Inc. (Tyler, TX). Dissection of the jaws took place under sterile conditions in a laminar flow hood and required ~20–30 min per jaw, as shown in Fig. 1. After first separating the upper and lower jaw halves to reveal the joint capsule, the superior surfaces of the discs were exposed by delicately removing the connective tissue attaching them to the temporal bone. Ligaments connecting the disc to the condyle were then severed using a scalpel to make the final disc separations. Any remaining retrodiscal tissue attached to the posterior disc band was carefully cut away. Discs with perforations or signs of disc injury were discarded. Baseline data were then taken off the initial disc dimensions in both the anteroposterior and mediolateral directions using a digital caliper.

2.2. Decellularization process

The porcine discs were divided into three sample sets of six TMJ discs each and each set was immersed in 250 ml of the appropriate decellularization solution: 1% (w/v) SDS, 1% Triton X-100, or a 25% acetone/75% ethanol (vol.%) combination. The sealed 500 ml capacity glass bottles containing the discs and the three solutions were agitated at 100 rpm. on a horizontal shaker plate for 24 h at 25 °C. After decanting the decellularization solutions, the discs were washed five times for 1 h each in phosphate-buffered saline (PBS) to remove the residual chemicals. Measurements of both the anteroposterior and mediolateral disc dimensions were recorded and subsequently compared to those of the freshly dissected discs. The discs were then stored in 0.15 M PBS (pH 7.4) at 4 °C until use.

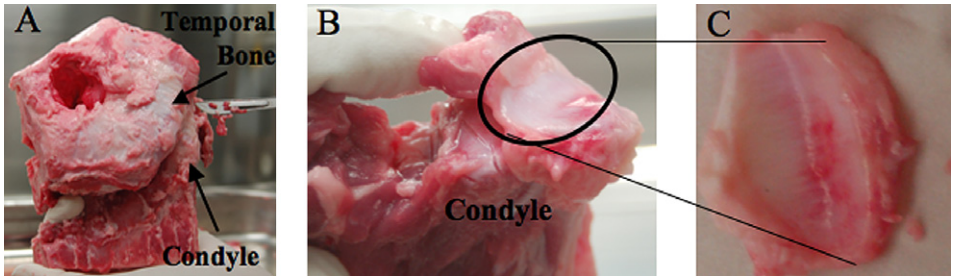

Fig. 1. TMJ disc dissection process. Porcine jaws were separated into the upper and lower joint compartments using a scalpel (A). Connective tissue attaching the disc to the temporal bone was removed, revealing the disc connections to the condyle (B). Ligaments connecting the disc to the condyle were severed to separate the disc (C).

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