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Technical Note

An effective procedure to create a speckle pattern on biological soft tissue for digital image correlation measurements

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ABSTRACT

Creating a speckle pattern on biological soft tissue, which would be suitable for digital image correlation measurements, is challenging. Speckle patterns should neither cause or require sample dehydration, nor alter the mechanical response, but they should adhere to the tissue surface and withstand large deformations.

A two-step procedure has been implemented to create a highly-contrasted pattern. It requires staining of the tissue with methylene blue solution to obtain a dark background and airbrushing the surface with paint to create white speckles. This study evaluated the effectiveness of the proposed procedure and whether the pattern creation had any effect on the elastic response of soft tissue.

Forty porcine collateral ligaments underwent three series of cyclic tensile tests to a nominal elongation of 10% for 30 cycles. The specimen stiffness was calculated from the load-elongation curve collected during the last 10 cycles. One side of 20 ligaments was blue stained between the first and second test series, and white patterned between the second and third test series. During the last series, ligament surface images were also acquired and elaborated using the digital image correlation technique. The other 20 ligaments were untreated.

The data show a small non-significant upward trend in stiffness in treated as well as in untreated ligaments (maximum increase of 1.7%). The 'successfully-correlated area' of the stereo-visible ligament surface was on average 96%, i.e. small parts of the 'stereo-visible area' were lost during computation.

The described procedure is an effective method to create a pattern on biological soft tissues.

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1. Introduction

Digital image correlation (DIC) technique has become popular to investigate the mechanical properties of biological soft tissues (Gao and Desai, 2010; Sutton et al., 2008). The DIC technique can monitor the displacement fields on the visible surface of a specimen, overcoming the main limitation of mechanical or optical transducers i.e. tracking only one-dimension elongation between two levels (Abramowitch et al., 2003; Kusma et al., 2008; Manoogian et al., 2009; Öhman et al., 2009; Schatzmann et al., 1998). This is a great advantage since biological soft tissue samples are neither regular in shape nor homogeneous and elongate non-uniformly (Lake et al., 2009; Sopakayang, 2013; Woo et al., 1983).

The DIC technique relies on tracking of unique details visible on the specimen surface (Schreier et al., 2009). Therefore, a highly-contrasted stochastic speckle pattern must be created on the specimen surface. Since DIC measurements are strongly affected by the quality of the speckle pattern (Barranger et al., 2010), the latter has attracted the interest of many authors (Barranger et al., 2010; Crammond et al., 2013; Hua et al., 2011; Lecompte et al., 2006; Pan et al., 2010).

If the creation of the surface pattern is not a trivial step for DIC measurements on standard specimens, the creation of a pattern on biological soft tissue surface can be challenging when a natural texture is not already present, such as in the liver tissue (Gao and Desai, 2010). Surface preparation of the sample must be performed quickly without tissue dehydration to avoid any changes in the mechanical properties (Haut and Hautt, 1997). Additionally, the surface pattern must not alter the soft tissue behaviour and must withstand large deformation (10–30%) (Mattucci et al., 2012; Ticker et al., 1996).

Several techniques have been proposed to create speckle patterns on biological soft tissue surface. These consist in the use of powders (Myers et al., 2010), powder ink mixed into a gelatinous substance (Thompson et al., 2007), and paint (white paint for background, black paint for speckles) (Libertiaux et al., 2011; Moerman et al., 2009), which must be distributed, applied or sprayed on the tissue sample surface (Zhang et al., 2002). The use of spherical particles of controlled dimensions guarantees the desired speckle size. Unfortunately, the powder adhesion to the specimen surface is not reliable with time and pattern detachment may locally affect DIC measurements. This problem can be overcome using gelatinous substances. This technique requires the application of a thin and homogeneous film on the specimen surface. While it has been successfully deployed on small flat specimens (Thompson et al., 2007), it is not feasible with soft, irregular in shape specimens. The use of paint avoids these complications. However paint coating, necessary to create a black or white background, may alter the tissue mechanical response. This problem has been rarely addressed in experimental studies. In such cases only qualitative checks were reported (Libertiaux et al., 2011), while the evaluation of the quality of the pattern (speckle size) obtained on the tissue surface has been generally neglected with a few exceptions (Lecompte et al., 2006; Ning et al., 2011). Even when the desired speckle dimension is achieved, paint film delamination and crumbling may occur under large tensile deformation (Schreier et al., 2009). Therefore alternative solutions are being sought after.

We present here an easy and practical technique, which has been implemented to create a stochastic pattern on the soft tissue surface of collateral ligaments trying to overcome the above mentioned problems. The procedure involves the use of saline solution containing 1% methylene blue to stain the tissue surface dark blue without the need of a coating film. Then, the speckle pattern is created on the sample by spraying small dots of white paint directly on the tissue surface with an airbrush. The aims of the study were: firstly, to verify whether the pattern creation had any effect on the elastic response of the soft tissue; secondly, to evaluate the effectiveness of the proposed procedure.

2. Materials and methods

2.1. Material

Porcine collateral ligaments were selected as model for this study. Knee samples were collected from a local slaughterhouse. Pig knees were excised with 5 cm of distal femur and proximal tibia. Immediately after harvesting, samples were wrapped with a saline solution soaked gauze, placed into a sealed plastic bag and frozen at $-20\,^{\circ}$C.

2.2. Specimen preparation

Each knee sample was thawed at room temperature. The preparation started when the specimen appeared thawed touching the external soft tissue with a finger. All unwanted soft tissues were removed or cut exposing the two collateral ligaments. Each collateral ligament was excised with both insertions requiring the resection of a selected portion of the tibia and femur. The proximal bone block of each ligament was clamped using a 3-degrees-of-freedom clamp. In order to align the ligament vertically, the clamp was oriented around two orthogonal horizontal axes. The clamp was lowered inserting the distal bone block into a mould made of high-density polyethylene. The mould had a horizontal square inner cavity open at the top. The mould was filled with polymethylmethacrylate (PMMA). The amount of PMMA was controlled reaching the upper part of the bone block. During curing, the bone block was manually pushed downwards in order to maintain the ligament in the desired position. When PMMA curing started, causing the viscosity to rise, the clamp was lightly raised. This operation leads to a more homogeneous stress distribution within the ligament. In fact, any unbalanced moment within the ligament would cause a rotation of the bone block within the curing PMMA. Once the polymerization was completed, the PMMA block was removed from the mould. The described procedure was repeated to embed the proximal ligament insertion into PMMA. In this case the PMMA block was clamped vertically in the 3-degrees-of-freedom clamp, therefore also the ligament was vertical. Finally, the edges and corners of the PMMA blocks closer to the ligament, were rounded using a portable rotary tool equipped with a high-speed cutter. The whole preparation took about one hour and half. To avoid