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J Dent Res DOI: 10.1177/0022034513494817

Tensile Characterization of Porcine Temporomandibular Joint Disc Attachments

APPENDIX

MATERIALS & METHODS

Specimen Preparation

With the joint capsule remaining intact, the TMJ disc-attachment complex was released from the fossa, and a condylectomy was performed. Excised joints were washed with phosphate-buffered saline (PBS, Sigma, St. Louis, MO, USA), wrapped in gauze soaked with PBS containing protease inhibitors (10 mM *N*-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride, Sigma), and frozen at -20°C until testing. After being thawed, TMJ attachment and disc specimens were isolated (Figs. 1a, 1b, main paper) and verified to be grossly normal.

Tensile Testing

We used the disc's intermediate zone as a control by comparing the stiffness with values reported in the literature (Beatty *et al.*, 2001). Testing specimens were excised from the region of interest and were tested in only one direction. Dog-bone-shaped specimens were approximately 12 mm in length (n = 9 for each region and direction), with the region of interest within the gauge region. Within the gauge region, the samples were 1.9 mm ± 0.5 mm in width and 1.7 ± 0.4 mm in thickness. The initial gauge length of dog-bone-shaped specimens was considered to be the grip-to-grip length. Tensile testing was conducted with a Test Resources 840L (TestResources, Inc., Shakopee, MN, USA).

The strain rate of 1%/sec was used to approximate the tissues' inherent viscoelastic material properties. However, this is below what is seen physiologically (Herring and Scapino, 1973; Liu and Herring, 2000).

By plotting the raw data of stress on the y-axis against strain on the x-axis, we generated stress-strain curves, and Young's modulus, UTS, maximum strain, and toughness were calculated based on the following: Young's modulus, which is the slope of the linear region of the stress-strain curve, is a measure of the tissue's stiffness, or resistance to stretching. UTS is the maximum stress the tissue is capable of sustaining prior to breaking. Maximum strain, the strain corresponding with UTS, is the total, normalized deformation the tissue experiences up to maximum stress. Toughness is the energy, calculated as area under the curve using the trapezoid rule up to maximum stress, that the specimen absorbs before failing.

Macroscopic Characterization

For SEM imaging, rectangular specimens were cut (2 mm x 2 mm x 6 mm) and fixed in 3% gluteraldehyde (Sigma) in cacodylate buffer for 4 days at 4°C. Specimens were dehydrated in ascending ethanol exchanges, and cryofractured in 100% ethanol. Specimens were lyophilized for 15 min, mounted, and imaged (Phenom G2 Pure, Eindhoven, Netherlands).

APPENDIX REFERENCES

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