





# Abstract

## Ovine Model as a Temporomandibular Disc Substitute: Characterisation and the Outcomes of Freezing Storage<sup>†</sup>

Daniela Trindade<sup>1</sup> , Cecília R. C. Calado<sup>2,3</sup> , Nuno Alves<sup>1,\*</sup>  and Carla Moura<sup>1,\*</sup> 

- <sup>1</sup> Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, 2030-028 Leiria, Portugal; daniela.trindade@ipleiria.pt
  - <sup>2</sup> ISEL—Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, 1959-007 Lisboa, Portugal; cecilia.calado@isel.pt
  - <sup>3</sup> CIMOSM—Centro de Investigação em Modelação e Optimização de Sistemas Multifuncionais, ISEL—Instituto Superior de Engenharia de Lisboa, 1959-007 Lisboa, Portugal
- \* Correspondence: nuno.alves@ipleiria.pt (N.A.); carla.moura@ipleiria.pt (C.M.); Tel.: +351-244-569-441
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**Keywords:** temporomandibular joint disc; ovine model; freezing time storage; compression properties; biochemical composition



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The temporomandibular joint (TMJ) is a ginglymoarthrodial joint that comprises a fibrocartilaginous disc that is highly predisposed to suffer from trauma or degenerative events, leading to disorders in the condyle–disc complex. The use of decellularized tissues has attracted interest in the tissue engineering (TE) field as an intact extracellular matrix can be obtained [1]. To this end, the ovine model was the first choice for a TMJ TE approach as they are easy to obtain, inexpensive and present an easily accessible surgical site [2]. When biological materials are used, their conservation should be taken into consideration, as sometimes it is not possible to test them immediately after extraction. With this, the present study aims to characterise the ovine disc, and determine its maximum freezing-time storage without alterations in its morphological and compression properties and biochemical composition. For this, two storage conditions were tested: (i) freezing at  $-20\text{ }^{\circ}\text{C}$  in phosphate buffered saline (PBS) solution and thaw at  $4\text{ }^{\circ}\text{C}$  (PBS +  $4\text{ }^{\circ}\text{C}$ ) and (ii) wrapping the discs in PBS embedded gauze and freezing at  $-20\text{ }^{\circ}\text{C}$  followed by thaw at room temperature (RT) in PBS (Gauze + RT). Moreover, different time intervals were assessed: 1, 7 and 14 days. Results showed that the native disc presented a thickness of  $1.62 \pm 0.674\text{ mm}$ , weighted  $0.385 \pm 0.029\text{ g}$  and had a compressive modulus of  $2.36 \pm 0.072\text{ MPa}$ . Regarding biochemical composition, collagen content was higher in the central zone, whereas glycosaminoglycans were higher in the lateral and posterior zone. After performing both storage methods, morphological characteristics were minimally altered, but the biochemical content was significantly affected. After the 14-day experiments, there was an increase of about 30% in the compression modulus for Gauze + RT when compared to the fresh native disc, while for the PBS +  $4\text{ }^{\circ}\text{C}$  no changes were observed. This study determined that freezing the ovine discs may lead to changes in the native properties, indicating that when it is necessary to store them, these changes should be taken into consideration in future studies.

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