Ultrastructural and matrix evaluation of morpho-functional age-related changes in dog meniscus

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INTRODUCTON

Menisci are essential structures for the knee joint. Different attempts were made trying to replace or regenerate the meniscus after its tear, but the perfect solution is still far away. A better knowledge of the physiologic development of this structure through time could be useful to understand its behavior in the light of the tissue bio-engineering. In this study, the changes in canine meniscal morphology were evaluated to assess how it varies among diverse age stages.

OBJECTIVE

The aim of the study is the evaluation of morphological and biochemical changes that occur during the physiological development of meniscus in dog.

MATERIALS & METHODS

The fibers arrangement and matrix deposition in canine menisci from neonatal (died at birth), 10-days, 30-days and adult dogs, dead for causes not related to the present study, were evaluated by means of histochemistry (safranin-O and Sirius red staining), polarized light microscopy, immunofluorescence (collagen I and II) and Scanning Electron Microscopy (SEM). Moreover, quantitative measurements of glycosaminoglycans (GAGs), DNA and GAGs/DNA ratio were performed.

RESULTS & DISCUSSION

The "knotty" structure of neonatal meniscus is probably due to balls of collagen fibres that are not completely stretched until the 30-days stage (Fig 1). The stretching of the fibres starts from the inner portion that is probably the first and the most compressed zone.
Safranin-O staining shows how matrix composition vary during growth. Neonatal meniscus is characterized by a huge number of elongated cells (fibroblast-like) and GAGs, features that characterized a still afunctional tissue. With growth, more and more cells assumed a rounded shape. The end-point of the maturation process is represented by the adult meniscus: it is characterized by almost only rounded cells (fibro-chondrocyte-like), in small number, and surrounded by matrix (Fig 1). Nevertheless, 10-30 days interval could be considered the starting point of the meniscus specialization and maturation.
Fibres arrangement starts like balls of collagen fibres that follow a disorganized pattern in the neonatal meniscus (Fig 1). In 10-days meniscus, these balls of fibres tend to disappear starting from the meniscus' inner portion, in association with an initial organization of the fibres according to the longitudinal and radial axes of the meniscus. The organization of fibres network is almost complete at 30-days of life, when all the fibres follow the two main axes of the meniscus and show a well-organized disposition, as seen in adult meniscus.
Through the double immunofluorescence it is possible to recognized different aspect of maturation (Fig 3). Neonatal meniscus shows almost only collagen type I fibres. Collagen type I and II co-expression starts at 10 days (yellow) and become more evident in 30-days meniscus in which even a differentiation of the inner and the outer zone starts. The same differentiation persist in adult meniscus that is characterized by a frankly fibro-chondrocitic-like cellular phenotype.
Biochemical analysis confirmed that cellularity decrease over the time starting from neonatal to adult (Fig 3). The same decreasing trend is observed in GAGs deposition. Even if 30-days meniscus present a lot of common characteristics with the adult one, the GAGs/DNA ratios show how the latter is the only that present a maturely functional tissue in which a small number of cells is able to produce a matrix rich of GAGs.

CONCLUSION

Meniscal structure changes during growth. The starting point is represented by the neonatal tissue, rich of immature cells and with poor expression of matrix components. The end-point is the adult tissue, characterized by phenotypically mature cells, which assure a functional matrix deposition. Ten-thirty days interval seems to be the turning point of this developmental process. This work highlights how dog meniscal structure changes its morphology among different age stages; this fact may suggest a role of the biomechanical forces, physiologically acting on meniscus, in the development of its ultimate shape and functions. The knowledge of the developmental process of a structure has a capital importance to comprehend its physiologic anatomy and function.

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RESULTS

Fig 1

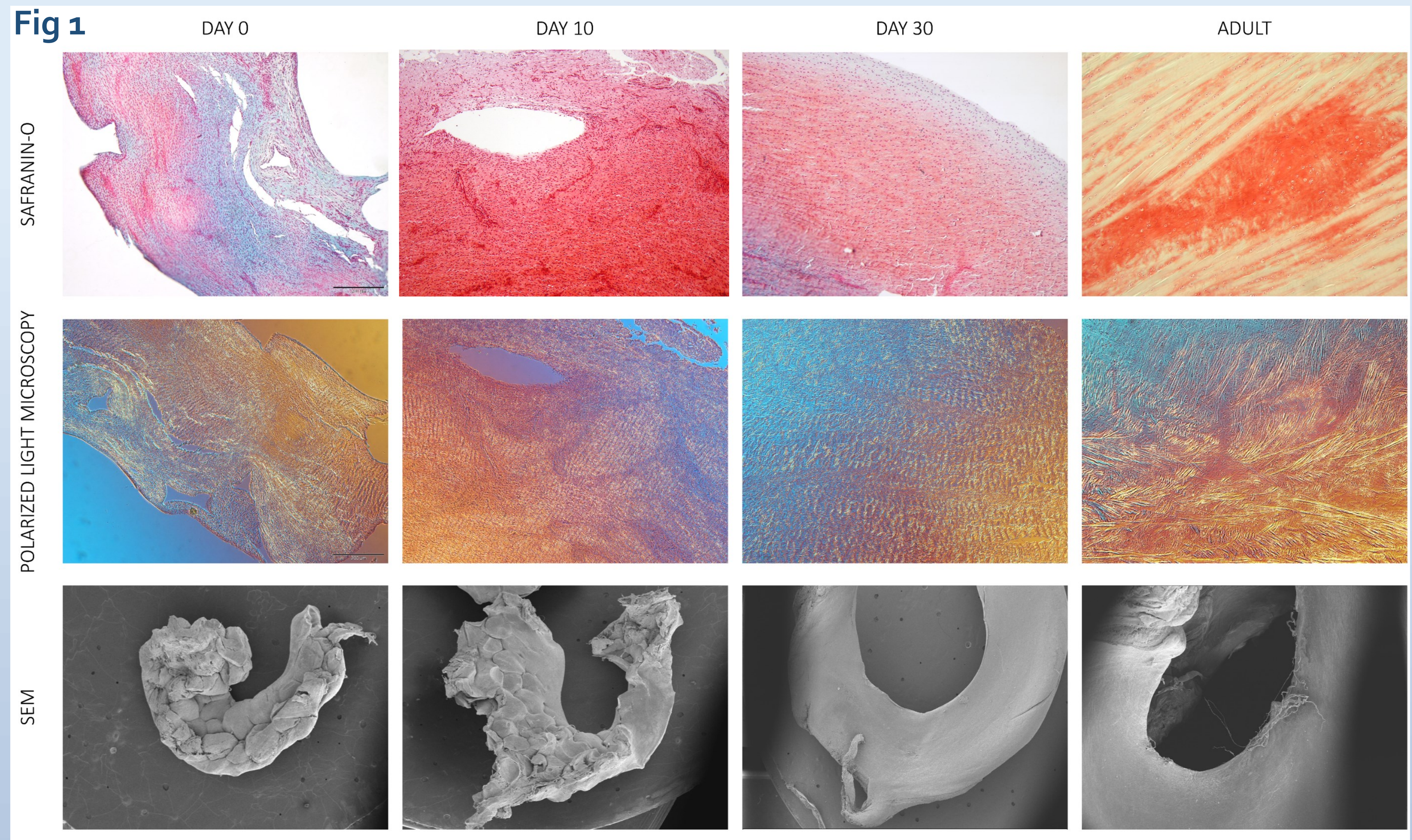


Fig 2

BIOCHEMICAL ANALYSIS

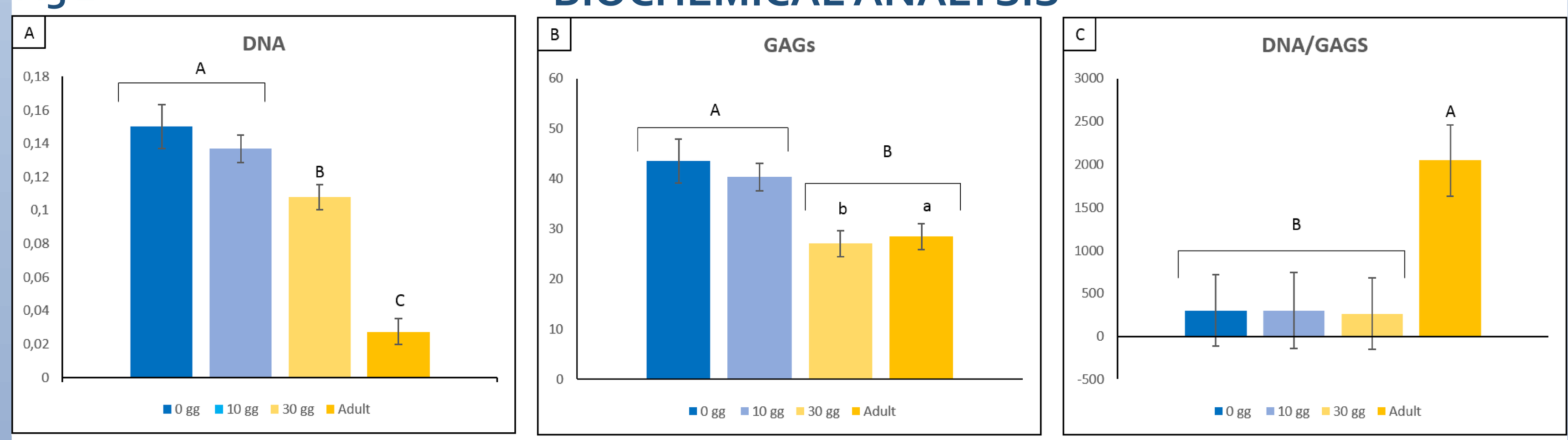
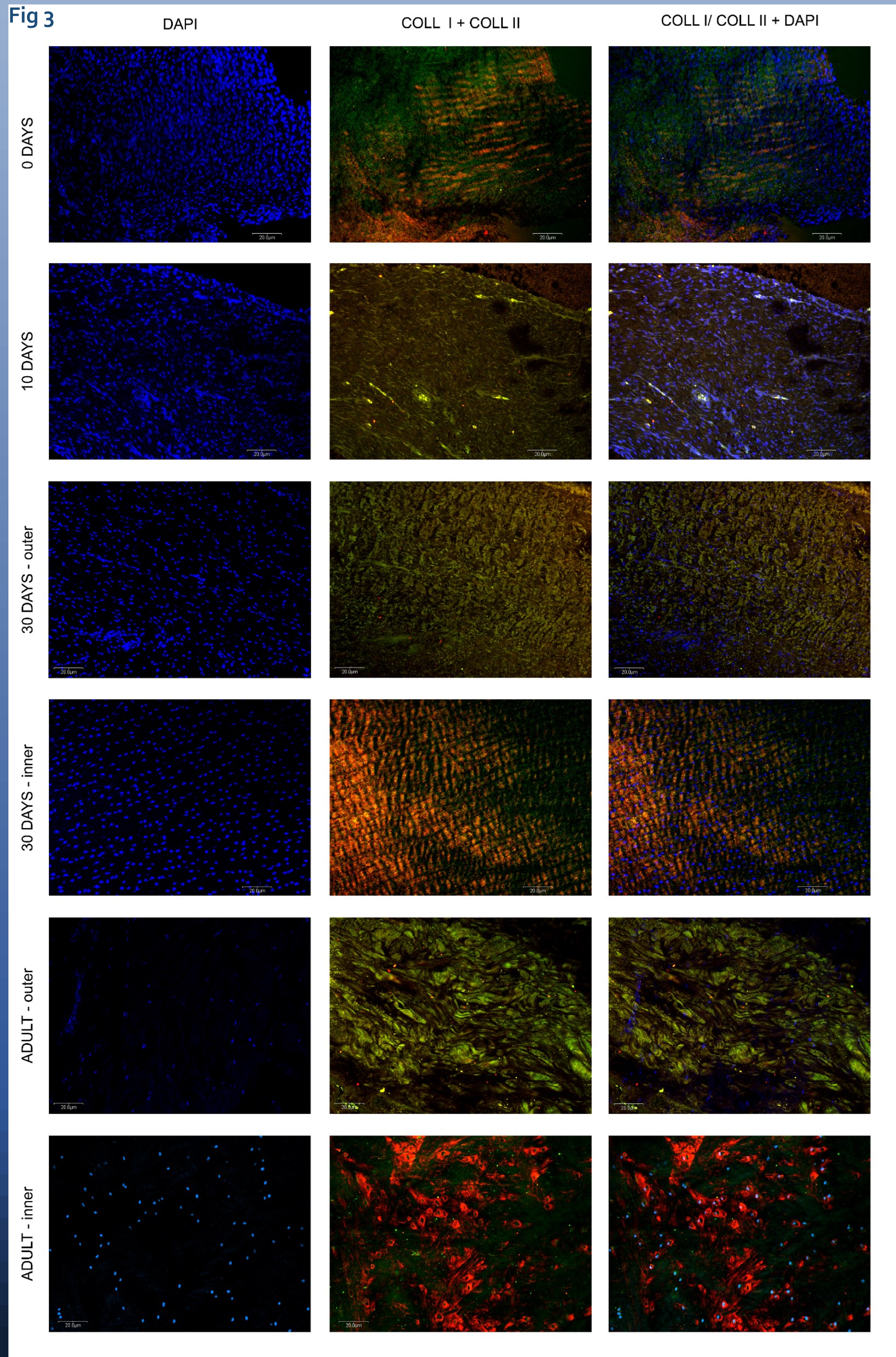


Fig 3



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