NEW INSIGHTS INTO MUTABLE COLLAGENOUS TISSUE

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Abstract

The biomimetic approach represents a new strategy pursued in the field of human regenerative medicine, since existing biomaterials lack the inherent adaptability of natural tissues; in particular, they do not truly mimic the dynamic microenvironment of tissues and organs.

Echinoderms are a good example of this ability, since they possess dynamic connective tissues called Mutable Collagenous Tissues (MCTs), able to undergo changes in their mechanical properties (stiffness, tensile strength and viscosity) in a short physiological time scale. This phenomenon is called mutability, and is initiated and modulated by the nervous system, especially by secretions of a specific cell type, the juxtaligamental cells (JLCs). Several studies reveal that MCTs are one of the key elements of echinoderm regenerative capacities, since they provide a dynamic ECM with an optimal growth-promoting environment for tissue repair and regeneration. However, the mechanisms that are behind the capabilities of MCTs to assume distinct mechanical states are still enigmatic. Thus the main aim of this work was to contribute for the understanding of those mechanisms.

The model studied was the compass depressor ligament (CDL) of sea urchin Paracentrotus lividus. The first part of this work consisted on the investigation of the CDL ECM key-components, including fibrillar proteins but also the JLCs. Their structure and arrangement were studied in order to understand how natural MCTs actually function. Electron microscopy techniques were used to obtain a three-dimensional view of the CDL architecture at the micro- and nano-scales, and to clarify the micro-organization of the ECM components when the tissue changes from the compliant to the standard state or from the standard to the stiff state. With this investigation we expand the current knowledge of the relationship between organization of CDL ECM and its different mechanical states. The biochemical changes that the CDL undergoes during its reversible tensility were investigated in detail. Confocal Raman microscopy, and Fourier Transform Infrared spectroscopy were used to investigate the possible similarities between CDL ECM and mammalian ECM components. The possible remodelling of a new ECM, as well as the contribution of water to CDL mutability, were hypothesized and evaluated, since these phenomena normally occur in mammalian adaptable connective tissues (such as the uterine cervix). We found that the fibrillar collagen has strong similarities with collagen type I and that glycosaminoglycans (GAGs) are from the sulphate family. Also, we concluded that CDL mutability involved subtle adjustments of protein components and tissue hydration, most likely without synthesis of a new ECM.

In view of the fact that mammalian ECM homeostasis is balanced by local protease activity involving matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), the potential function and involvement of MMPs in CDL mutability was also investigated. This work has provided the first evidence that MMPs may be involved in the mechanism by which echinoderm MCT undergoes changes in tensile properties. Gelatin zymography has revealed the presence of a consistent pattern of MMP activity that varies quantitatively according to the mechanical state of the ligament. Biomechanical results also demonstrate that MMPs are involved in CDL mutability, since an increase in CDL stiffness occurs upon stimulation with an MMP inhibitor. Similarly to mutability the stiffening action of the inhibitor was reversible.

Another major contribution of this work was the integrated morphological, biochemical and biomechanical investigation that was performed in CDL, comparing different mechanical states that mimic the mutability of the tissue in vivo. The acquired knowledge regarding to CDL structure, biochemistry and organization, as well as the contribution to understanding the mechanisms that promote such

dynamic and reversible environment, may be inspiring for biomaterials scientists. As these structures are commonly present at the autotomy planes of echinoderms, enhancing tissue regeneration, the knowledge obtained in this work may open new and exciting strategies in regenerative medicine.