EFFECTS OF "IN SITU" VITAMIN E ON FIBROBLAST DIFFERENTIATION AND ON COLLAGEN FIBRIL DEVELOPMENT IN THE REGENERATING TENDON.

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To study the effects of vitamin E on a regenerating tendon, a partial tenotomy was performed in a rabbit Achilles tendon. A 2-hydroxycetyl metacrylate (HEMA) polymer hydrogel was used for the "in situ" administration of vitamin E. HEMA was used as an adherent substratum as it favours fibroblast migration and facilitates the "in situ" administration of a certain vitamin E dose. The regeneration front of vitamin E-treated and non-treated (controls) samples was studied and compared 10, 20 and 30 days after tenotomy.

The tendon was fixed in 2.5% glutaraldehyde - 2% tannic acid, at pH 7.33, 4°C for 8 hours, postfixed in 1% osmium tetroxide for 1 hour and embedded in araldite. Ultrathin sections obtained were stained with lead citrate and observed under a transmission electron microscope.

"In situ" vitamin E-treated sections show an intense fibroblast proliferation ("activated fibroblasts") when compared to controls. Activated fibroblasts are needed for the urgent collagen fibril biosynthesis taking place in the regenerating tendon. At an ultrastructural level, intracellular collagen fibrils and an increased collagen protein biosynthesis are observed.

HEMA hydrogel contributes to the reorganization of the regenerating area in the tendon as a scaffold for fibroblasts in migration with vitamin E increasing fibroblast activity resulting in an acceleration of nascent collagen fibril biosynthesis.

Vitamin E-treated samples contain more collagen fibrils than controls. In vitamin E-treated samples, collagen fibrils appear earlier with mitosis being frequently observed among fibroblasts. The angiogenic response observed is significantly more important than that in controls but there are less macrophages and inflammatory cells in vitamin E-treated samples.

Vitamin E seems to shorten the time needed for tendon regeneration acting on fibroblast proliferation and secretion of nascent collagen fibrils.

Under the electron microscope, activated fibroblasts show non-membrane-bound large intracellular spaces containing either isolated or parallelly arranged collagen fibrils. With these images we support the hypothesis of intracellular collagen biosynthesis. These large intracellular spaces are limited by a fine dense granular substance and procollagen filaments that enter the lumen of the spaces to constitute, by parallel apposition, 30 nm-thick nascent collagen fibrils that show a 67 nm periodical striation. Thus, the long nascent collagen fibrils originated remain in the non-membrane-bound large intracellular spaces. One, two or more collagen fibrils may be observed in each of the spaces. Once their synthesis is completed, collagen fibrils leave the cell via plasma membrane irregularities into the extracellular space. In vitamin E-treated samples, this macro-exocytosis process is enhanced by the activated fibroblast to meet the urgent demand for collagen fibrils in the regenerating tendon.

References


Figures

Figure 1. Activated fibroblast. Nucleus (N). Extracellular collagen (C), intracellular collagen fibrils (arrows) leaving the fibroblast (*). Electron-dense bodies (e.b.) x6300.
Figure 2. Activated fibroblast. Nucleus (N). Rough endoplasmic reticulum (RER). Fine dense granular substance (*). Intracellular collagen fibrils (arrow). x3150.