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CALCIUM ION RELEASE FROM ELECTRORESPONSIVE BIOMIMETIC POLYMERS

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Abstract. Tissue repair is a natural biological process involving molecular intercellular communication. Investigation and of this process are essential for elaboration of smart materials and devices for effective injury treatment. In this study, biocompatible calcium containing polymers (calcium alginate and calcium polyacrylate) have been designed as prototype materials for controllable release of calcium ions, the active species of intercellular communication on human dermal fibroblast cells (HDF). The controllable release of calcium ions has been investigated under applied DC voltage in the range -5 to 5 V.

Introduction. Wound healing is known to be accompanied and supposedly controlled by intercellular communication. However, the mechanisms of the intercellular communication, signaling, and response are still poorly understood. Understanding of such mechanisms would be essential for developing new materials and approaches for effective injury treatment. The process of tissue gap repair has been widely addressed in literature. However, the functional relationship between the waveform of the signaling calcium concentration gradient and the evolution of the cells ensemble has not been established yet. In particular, the effect of the frequency and the amplitude of the gradient oscillation is of the primary interest addressed in this study.

Main part. To investigate the mechanism of intercellular communication by calcium waves, an artificial biomimetic device has been elaborated. This device consists of a glass substrate covered by a conductive layer of fluorine doped tin oxide (FTO). As the top layer, the film is coated with a polymer material containing weakly bonded calcium ions. The device is placed in a container with HDF cells. The external voltage is applied between an electrode dipped into the extracellular environment and the bottom FTO layer, so that most of the potential drops across the polymer film and stimulates the release of calcium ions. As the biomimetic calcium releasing polymers, calcium alginate and calcium polyacrylate have been synthesized and investigated.

1 wt.% of water solution of sodium alginate and 1 wt.% of methanol solution of polyacrylic acid were coated on the FTO by spin coating method. Then films were immersed in a saturated calcium chloride solution to obtain calcium alginate and calcium polyacrylate. The thickness of the obtained films was evaluated by atomic force microscopy in the range of 10-30 nm. Their biocompatibility was assessed by the MTT assay in HDF cells and demonstrated the viability of 85-90%. The electrical signal was applied on the polymer film using a SourceMeter Keithley 6430. The release of calcium ions was detected by spectrophotometric method using alizarin red S as an indicator. The external voltage was applied with various amplitudes ranging from -5 to +5 V and the rectangular pulse durations ranging from 5 min to 2 h. The resulting oscillation of calcium ions was observed in the range of 0 to 6.2 mmol/L.

Conclusions. A series of alginate-based polymers with different concentration of calcium has been synthesized and investigated as the calcium releasing biomimetic films. MTT assays have demonstrated high biocompatibility of obtained polymers. Viability of HDF cells was up to 90%. Thickness of polymer films varied from 10 to 30 nm. The experiments showed that the calcium ion release occurs under applied voltage ranging from 0.1 to 1 V resulting in a rise of the calcium concentration up to 1.5 times.

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