PREPARATION OF FIBRIN-BOUND THROMBIN MEDIATED FIBRIN ASSEMBLIES

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Conversion of fibrinogen into fibrin (fibrin assembly) is mediated by thrombin, which cleaves two pairs of fibrinopeptides, fpA and fpB, from the fibrinogen Aa and Bb chains, respectively. It is well established that fibrin assembly occurs in two stages starting with the cleavage of fpA, whose removal triggers formation of two-stranded protofibrils, followed by the removal of fpB, which enhances lateral association of protofibrils into thicker fibers. It is also known that protofibril formation accelerates removal of fpB. Non-substrate interaction of thrombin with the central part of fibrin(ogen) seems to play an important role in these processes. Fibrin network serve as a temporary extracellular matrix and promote wound healing. Cells migrate to the site of injury and bind specifically through integrin receptors to the fibrin network at which they can proliferate and differentiate. Fibrin network is degraded by proteolytical activity of cells and a new extracellular matrix is formed.

We developed a novel technique of fibrin gel formation (1) based on the catalytic effect of fibrin-bound thrombin (2) and on the nature of thrombin inhibitors. This technique is suitable for formation of attached thin fibrin network on artificial surfaces (Fig. 1a) without formation of fibrin network in bulk solution (Fig. 1b) and might be useful for coating of the inner surface of porous scaffolds designed for tissue engineering, without filling them with bulk fibrin clot, so that the space for cell migration and proliferation inside the scaffold can be preserved. Atomic force microscopy and scanning electron microscopy was used to study the morphology of fibrin assemblies, prepared using a technique mentioned above.

The gel morphology depended on the orientation of fibrinogen molecules in the initial adsorbed monolayer. Different combinations of thrombin inhibitors lead to various thicknesses of the fibrin gels. The average thickness of dried fibrin gel produced by catalytic action of thrombin bound to adsorbed fibrinogen/fibrin monolayer on ambient fibrinogen solution containing ATIII and heparin was 13nm (Fig. 2a). The average thickness of fibrin gel produced by catalytic action of surface bound thrombin was 40nm (Fig 2b) if the ambient fibrinogen solution contained only ATIII.

Coating substrate with surface fibrin gels supported growth of vascular endothelial cells on the surface (3). This technique has a potential application for coating inner surface of porous scaffolds without filling pores with a bulk fibrin network.

References:

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Figures:

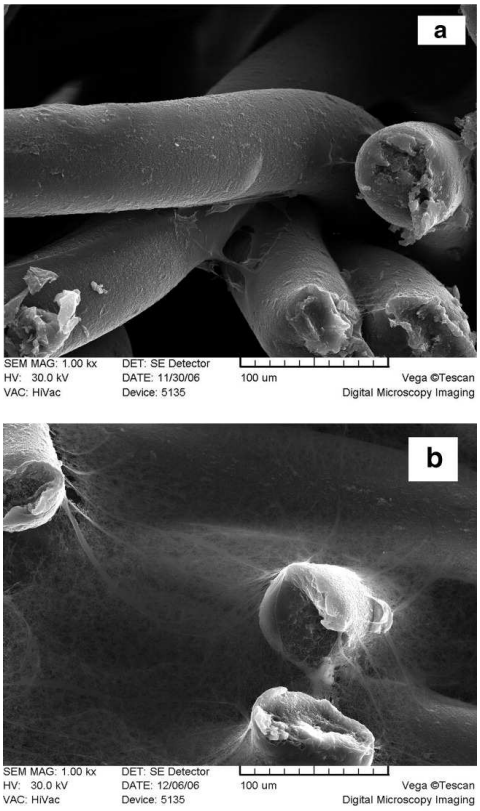

Figure 1. SEM of polylactide fibrous scaffold modified with fibrin structures. (a) The scaffold coated with thin surface fibrin gel produced by catalytic action of thrombin bound to adsorbed fibrinogen/fibrin monolayer on ambient fibrinogen solution containing ATIII and heparin. (b) The scaffold filled with bulk fibrin gel.

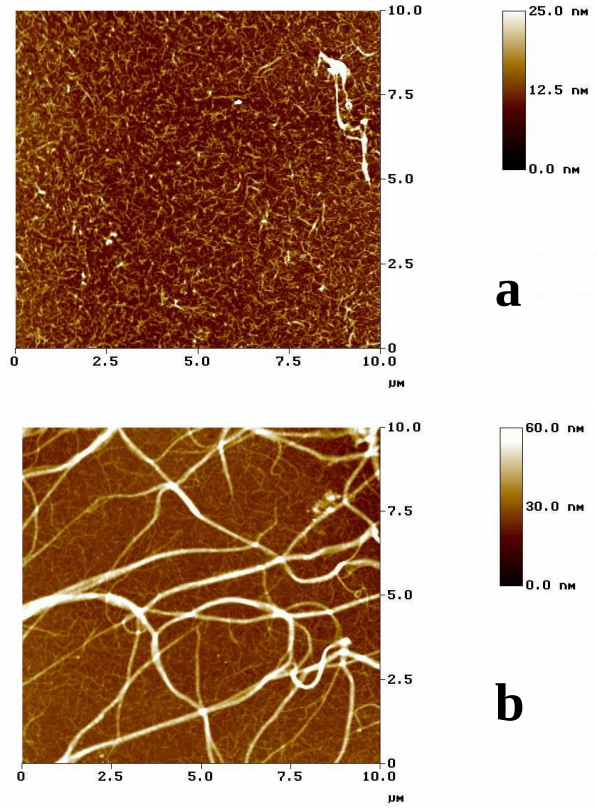

Figure 2. Fibrin gel produced by catalytic action of thrombin bound to adsorbed fibrinogen/fibrin monolayer on ambient fibrinogen solution containing (a) ATIII and heparin, (b) ATIII observed by AFM.

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