

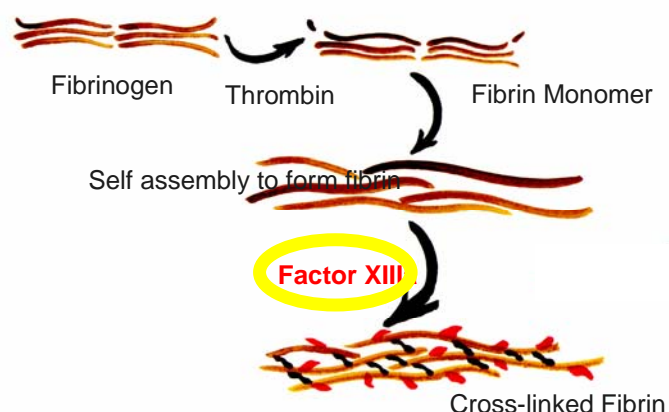
KUROS BIOSURGERY AG

Kuros Fibrin Matrices for Drug Delivery and Tissue Repair

Fibrin possesses unique properties that have been exploited in several biomedical applications. It forms spontaneously in a number of wound situations to provide closure and haemostasis, and it acts as a cell ingrowth matrix to promote tissue regeneration. Therapeutically as a surgical sealant, it is now available in clinical marketplaces throughout the world. Nevertheless, natural fibrin is limited in its ability to induce functionally optimal regeneration. Fibrin's usefulness could be extended if one could alter or enhance the biological character of the fibrin by incorporating within it the biological activity of other proteins and peptides. Kuros` technology seeks to do exactly this. The key feature is to endow fibrin sealant with morphogenic signals that are not naturally present within fibrin through incorporation of morphogenic proteins.

Formation of Fibrin Hydrogel/Matrix

The processes by which fibrinogen is polymerised into fibrin and by which this fibrin is subsequently degraded have been well characterised. Initially, the protease thrombin cleaves fibrinogen. Once the fibrinogen is cleaved, a self-assembly step occurs in which the fibrinogen monomers come together and form a covalently crosslinked fibrin network in the presence of Factor XIII, thrombin and a calcium source.



Proteins and peptide incorporated into the fibrin gels

The growth and regeneration of cells within their environment is controlled by the binding of peptides and proteins such as growth factors to cell surface receptors in the body to induce a biological response of the body. Kuros technology enables the incorporation of peptides, proteins and small molecules (“drugs”) into the fibrin hydrogel to engineer and tailor the bioactivity of fibrin to a specific need and to present the drugs in a manner that is consistent with their normal biological mode of action. Kuros has developed a number of different proprietary mechanisms which allow the incorporation of drugs directly or indirectly into fibrin gels.

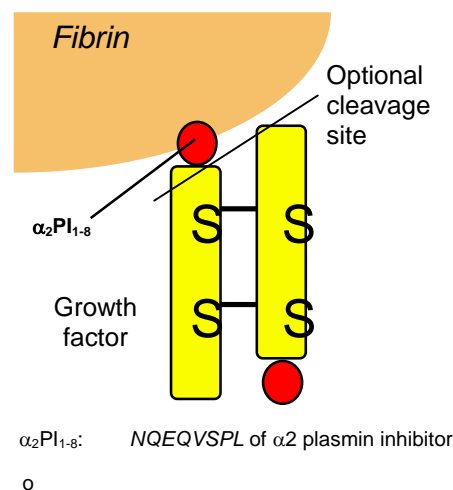
Of these mechanisms Kuros intensively pursues the direct incorporation of drugs into fibrin gels by way of covalently link the protein or peptide to the fibrin matrix (see scheme to the right).

The growth factor or peptide is modified by attaching an additional amino acid sequence at one end or both ends of the protein or peptide. The additional amino acid sequence is a

transglutaminase substrate domain, i.e. a domain which crosslinks into the fibrin network during its formation in the presence of Factor XIIIa, thrombin and a calcium source. The additional amino acid sequence is designed to contain an enzymatic cleavage site.

This technology enables the retention of a growth factor within a fibrin matrix during the process of wound healing. As part of the healing process, cells grow through the matrix and remodelling it. During this remodelling phase the cells release enzymes which cleave the enzymatic degradation site in the additional amino acid sequence thereby releasing the unmodified, wild version of the

Growth factor incorporation via enzymatic substrate linkers

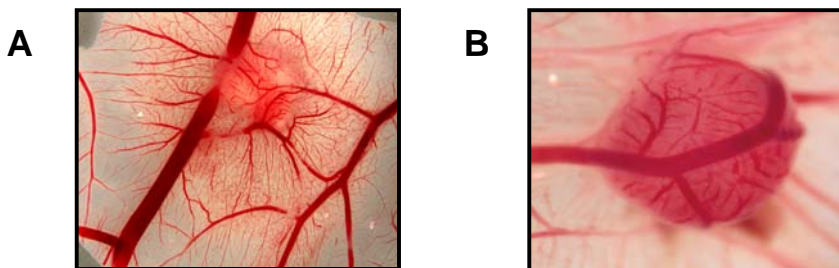


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growth factor or peptide. Subsequently the released growth factors and peptides trigger the body's own healing. By using this technology already low dose of the growth factors, peptides or other bioactive factors show efficacy since diffusion or burst release is avoided. The fibrin matrix allows other materials to be incorporated, like cells or materials that increase mechanical stability of the fibrin gel, like hydroxyapatite or calciumphosphate.

Kuros` fibrin technology enables a localized on-demand release of bioactive factors. The gels can be applied minimally invasively at the site of need in the body.

The figures below clearly illustrate the ability to localize cellular response using the Kuros technology. The figures show angiogenesis in the chick chorioallantoic membrane with a fibrin implant containing (A) diffusible VEGF in fibrin, (B) fibrin with a bound form of VEGF using direct incorporation.



Proof of Concept/Clinical Studies

Up to date Kuros has manufactured a series of fusion growth factors and peptides modified in the above described way. PDGF, BMP-2, VEGF121 and NGF were successfully manufactured with a transglutaminase substrate domain attached without any loss of their activity. The modified version of PDGF, "TG-PDGF", has been scaled up to GMP and is currently - covalently bound to a fibrin gel - employed in a 100 patients Phase I/IIa multi-centre clinical study for the treatment of venous foot ulcers. In a further Phase I clinical study a small fusion peptide, also covalently bound to a

fibrin gel, is used in the treatment of distal radius fractures. The efficacy of the modified version of BMP-2 could be shown by fusion of non-unions (arthrodesis) in over 40 dog patients.