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Enzymatic immobilization of VEGF to collagen-based medical products

INTRODUCTION

The successful integration of a medical implant in the host tissue requires sufficient supply with oxygen and nutrients. Adequate vascularization is therefore a major challenge in tissue engineering. Vascular endothelial growth factor (VEGF) is known to activate proliferation, migration, and survival pathways in endothelial cells through phosphorylation of VEGF receptor-2 (VEGFR-2). The incorporation of VEGF into biomaterials is therefore a reasonable strategy to induce neovascularization. Several techniques have been investigated, including encapsulation, electrostatic sequestration and chemical coupling of VEGF. However, incorporation of growth factors in collagen matrices is a crucial process due to the low thermal and mechanical stability of collagen, especially in humid or aqueous environment. The covalent immobilisation of VEGF using chemical cross-linking agents, like 1-ethyl-3(3-dimethyl-aminopropyl) carbodiimide (EDC) for example is a highly reactive process that has major impacts on the collagen structure and bulk properties. Furthermore, remaining residues of chemical crosslinking agents in the material can be toxic or induce an inflammatory reaction during the degradation in the body.

In this study VEGF165 at soluble and unsoluble collagen matrices was immobilized by tyrosinase-mediated enzymatic cross-linking. The effect of this immobilization strategy on VEGF signaling has been investigated.

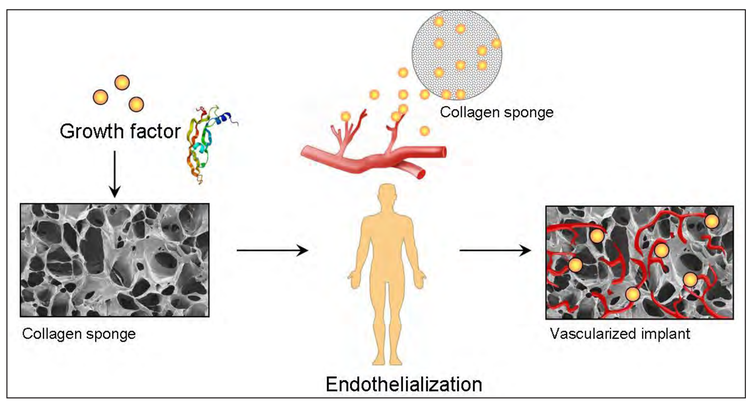


Figure. 1: Scheme of controlled biomolecule delivery to the implantation site

METHODS

ENZYMATIC COUPLING OF VEGF TO COLLAGEN

To prevent diffusion and uncontrolled release, VEGF has been covalently immobilized to collagen using the enzyme Tyrosinase from Agaricus Bisphorus under mild reaction conditions.

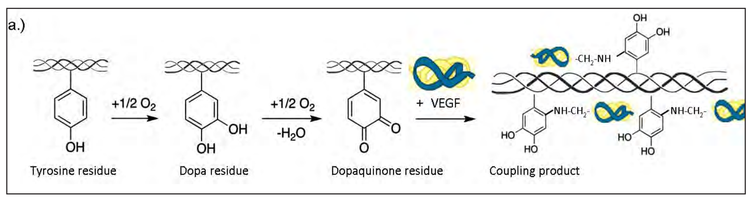
- Incubation of collagen with the enzyme and VEGF for 24 h at 30 °C
- Enzyme activity 200 U/mg collagen
- in 180 mM Potassium phosphat buffer, pH 7

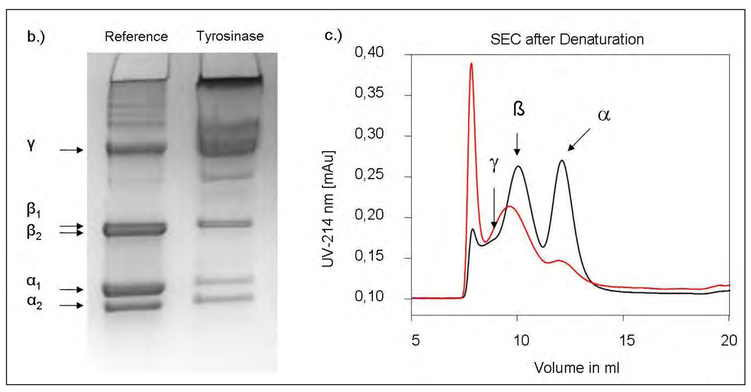
CHEMICAL PROPERTIES OF ENZYMATICALLY MODIFIED COLLAGEN

The incubation of collagen with Tyrosinase induces weak crosslinking of the collagen molecule and results in a slight increase in denaturation temperature of around 2 °C. Amino acid analyses indicated that 25 % Tyrosine residues of the collagen are affected by the treatment.

BIOLOGICAL CHARACTERIZATION OF VEGF ACTIVITY AFTER COUPLING TO COLLAGEN

The growth factor VEGF activates important signaling pathways involved in angiogenic events. It has been shown to stimulate endothelial cell mitogenesis and cell migration, and promotes cell survival.





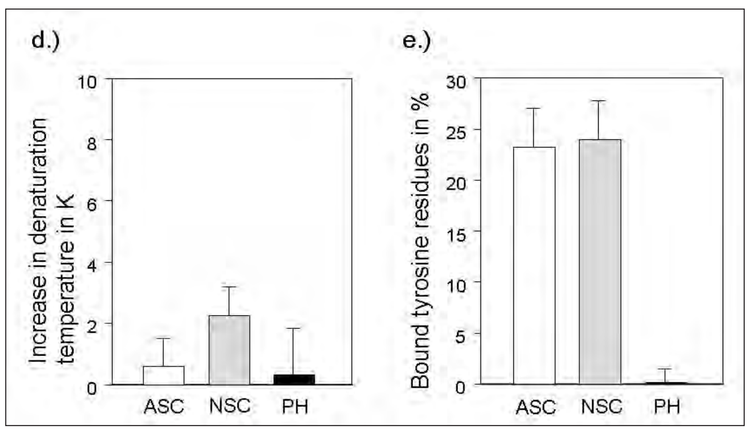


Figure 2. Impact of Tyrosinase treatment on collagen. a) One possible coupling mechanism induced by Tyrosinase. b) SDS-Page of collagen treated with Tyrosinase. c) Separation of collagen chains α, β and γ by size-exclusion chromatography. d) Changes of denaturation temperature after incubation with Tyrosinase. ASC = acid soluble collagen, NSC = non-soluble collagen, PH = porcine hide. e) Number of tyrosine residues involved in a chemical reaction.

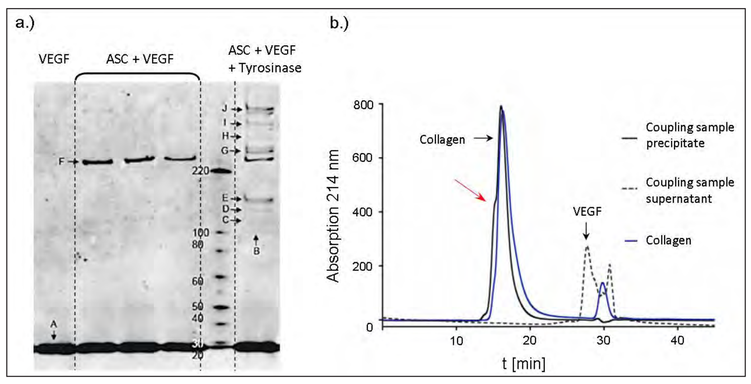


Figure 3. Detection of the collagen/VEGF coupling product. a) Immunochemical detection of VEGF in Western Blot. b) Analyses of the coupling product in size-exclusion chromatography.

BIOLOGICAL CHARACTERIZATION OF VEGF ACTIVITY AFTER COUPLING TO COLLAGEN

The growth factor VEGF activates important signaling pathways involved in angiogenic events. It has been shown to stimulate endothelial cell mitogenesis and cell migration, and promotes cell survival.

The biological activity of VEGF was analyzed in vitro. VEGFR-2 phosphorylation was investigated using enzyme-linked immunosorbent assay (ELISA). Receptor activation after incubation of HUVECs with collagen/VEGF coupling product was comparable to VEGF alone. Furthermore, the coupling product promoted the survival of HUVECs after strong starvation and increased cell proliferation as determined with XTT assay.

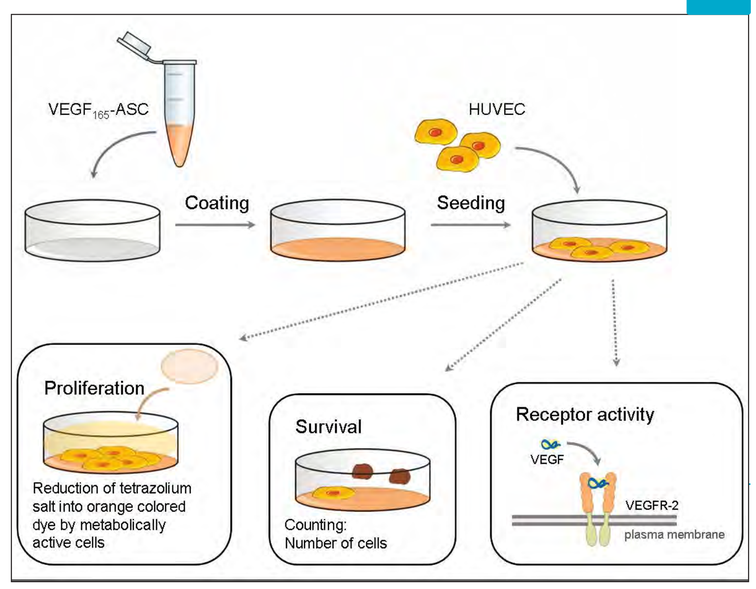


Figure 4. Experimental setup for VEGF activity testings.

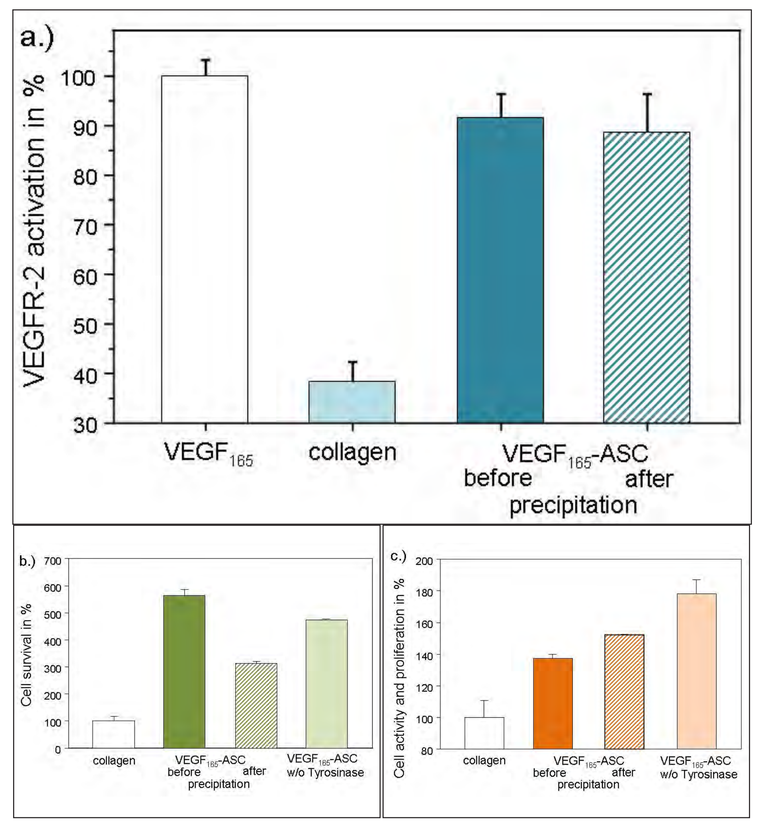


Figure 5. Biological characterization of VEGF activity. a) Detection of phosphorylated VEGF Receptor-2 in HUVEC cells with ELISA assay after incubation with collagen/VEGF coupling product. b) Survival of HUVECs after strong starvation. c) Proliferation and activity of HUVECs after 3 days of incubation with collagen/VEGF coupling product determined with XTT assay.

CONCLUSIONS

- Tyrosinase has weak impact on collagen
- VEGF remains within the collagen during precipitation and purification of collagen
- VEGF bioactivity stays intact after enzymatic coupling to collagen

Covalent binding of VEGF to collagen matrices using tyrosinases is a suitable concept to generate matrix-associated and soluble VEGF at the implantation site.

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