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Mini Review

Photochemical Tissue Engineering for Vascular Applications

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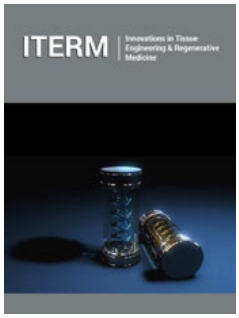

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Abstract

Photochemical tissue engineering is well suited for interventions applied to vascular diseases to control long term vascular remodeling. Photochemical tissue engineering methods described in this article utilize light absorption by a photosensitizer molecule producing reactive intermediates resulting in crosslinking of protein molecules at oxidizable amino acid constituents. A vascular application being developed utilizing photochemical tissue engineering is called Natural Vascular Scaffolding (NVS). NVS photochemically creates new covalent bonds between the natural structural components of the extracellular matrix to retain treatment-initiated shape changes, and to reset the hemodynamic stress on the cellular components. As a result of new protein crosslinks, the treatment increases the resistance of ECM proteins against enzymatic degradation and consequently reduces vascular inflammation. These changes to the ECM create long-term impact on the cellular response of tissue remodeling allowing prolonged patency of the vessels treated in vascular applications. These vascular changes have been shown to lead to benefits in arterial disease as well as in arteriovenous fistula creation using animal models and hold promise for clinical applications.

Keywords: Biologic scaffolds; Extracellular Matrix (ECM); Tissue engineering; Photochemical crosslinking; Natural vascular scaffolding; Tissue remodeling; Regenerative medicine; Photochemical tissue engineering

Abbreviations: ECM: Extracellular Matrix; PDT: Photodynamic Therapy; PTP: Photochemical Tissue Passivation; NVS: Natural Vascular Scaffolding; ROS: Reactive Oxygen Species; PTA: Percutaneous Angioplasty; AVF: Arteriovenous Fistula

Introduction

In recent years, the field of tissue engineering has made significant advancements, including innovative solutions for vascular applications. Success requires a coordinated multidisciplinary effort involving engineers, chemists, biologists, and medical researchers. The goal of tissue engineering is to apply biochemical and physicochemical methods to create or restore cellular environments for the purpose of functional tissue regeneration for biomedical purposes. The foundation of a newly engineered tissue is the scaffold provided by the extracellular matrix (ECM). The quality of the ECM determines the final structure and function of the tissue and organ.

Decellularized biological scaffolds are preferred over synthetic matrices, due to their ability to facilitate cell recruitment and to contribute to signal transduction maintaining the responsiveness of the tissue to changes in the microenvironment. The endogenous ECM consists of a combination of large structural proteins such as collagen, elastin, and fibronectin and other more functional macromolecules including proteoglycans and growth factors organized in a tissue specific three-dimensional network. The simultaneous stability and plasticity of this protein fiber mesh is critical for its role as a scaffold. The dynamic nature of the

ECM can be best influenced by the degree of crosslinking between the proteins building up the matrix. Different methods have been applied to alter the plasticity of the ECM. Chemical crosslinking approaches, such as the use of glutaraldehyde, produced ECM scaffolds which are unable to be degraded. These highly crosslinked scaffolds are unable to be remodeled by the surrounding biological environment and promote both inflammation and foreign body response.

Stabilization of the autologous, natural ECM for diverse biological application is best achieved by photochemical crosslinking. Photooxidative methods where a photosensitizer molecule is activated via the absorption of photons result in the formation of reactive intermediates at oxidizable amino acid sites in the protein. When two reactive amino acid sites are in close proximity a covalent bond can form, crosslinking protein molecules. The resulting linkage pattern is similar to that caused by enzymatic crosslinking. The photochemically treated tissue area, established by intermolecularly bonded proteins, provides resistance to enzymatic protein degradation without toxicity or compromised biocompatibility of the natural ECM scaffold [1,2].

Discussion

Types of photochemical approaches

Several different photochemical methods can be used for protein crosslinking. These reactions involve light absorption by endogenous (e.g., flavins) or exogenous (e.g., dye molecule) chromophores or photosensitizing reagents and the oxidation of reactive amino acids like tyrosine, tryptophan, histidine, cysteine and methionine. Crosslinking can be achieved either by direct reaction of the light activated photosensitizer with the protein or indirectly, with oxygen as an intermediate. With oxygen mediated linking, typically the photosensitizer excites oxygen from the ground state to the reactive singlet state, and singlet oxygen is then responsible for oxidizing and crosslinking the surrounding protein molecules. Photochemical crosslinking of proteins is nonspecific, and typically the actual crosslinking sites cannot be accurately located [2].

Riboflavin and Rose Bengal are the two most studied photosensitizers for medical applications. Their absorption spectra are different, for riboflavin the absorption maxima is in the UVA and blue (380-450nm) visible light range and for Rose Bengal it is in the green (550nm) visible light range, but their photophysical properties are similar [3]. During photoactivation the riboflavin is photolytically degraded resulting in several photoproducts [4]. Riboflavin photosensitization is an FDA approved clinical treatment for increasing cornea stiffness [5] based on photochemical protein crosslinking. Rose Bengal, unlike riboflavin, forms a ground state complex with collagen as aggregate, via non-covalent binding. Its medical applicability has been suggested [3]. A third photosensitizer, designed to meet attributes compatible with manufacturability for clinical use (i.e., coating on an angioplasty balloon), has a 4-amino-1,8 naphthalimide chromophore (10-8-10 Dimer) with a light absorbance maximum at 450nm. This photosensitizer participates in a photochemical reaction that leads to protein crosslinking as outlined in (Figure 1). Upon delivery to the treatment site, the 10-8-10 Dimer diffuses through the vessel wall. During the photoactivation step, 10-8-10 Dimer initiates crosslinking via mechanisms described by Keyes et al. [6]. Unlike Rose Bengal, it does not form complexes with the extracellular matrix substrate. Therefore, its diffusion through the tissue is not compromised. Its aqueous solubility also allows rapid clearance from the treated tissues.

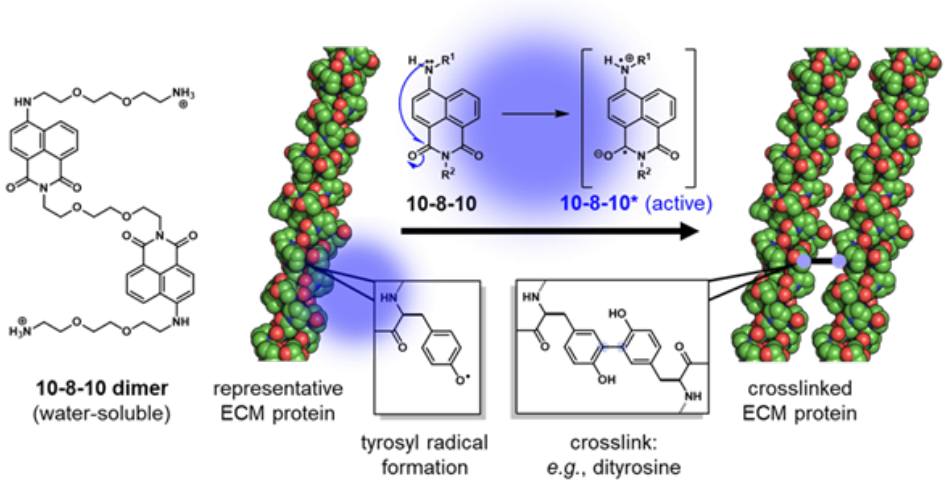

Figure 1: Proposed mechanism of 10-8-10 dimer photosensitized protein crosslinking. The 10-8-10 Dimer diffuses into the target tissue. The treatment area is irradiated by 450nm light (depicted by blue circle) to achieve crosslinking of ECM protein.

Note: tyrosine is provided as an example for amino acid crosslinks, other potential reactive amino acids involved in this reaction could include tryptophan, histidine, lysine, arginine, methionine, and cystine.

Clinical applications

Medical applications of photochemical treatment include photodynamic therapy (PDT), photochemical tissue passivation (PTP), and natural vascular scaffolding (NVS). These different applications utilize the same photochemical principle with differences in the application of light (e.g., wavelength), the photosensitizer molecules and the concomitant duration of exposure by the treatment in the vicinity of the target tissue. In the case of PDT, light activation initiates the formation of intracellular reactive oxygen species (ROS). The excess of ROS activates apoptotic pathways leading to cell death which is used as a cancer therapy [7]. PDT is also successfully used as an antimicrobial treatment for oral infections [8].

PTP is generally described as a reduced photochemical treatment of tissues with decreased inflammation and mostly imparting increased stiffening of the treatment exposed tissues, such as the cornea. The increased mechanical strength is achieved by prolonged exposure to the photochemical cross-linking process. NVS is the least invasive photochemical approach [9]. Structural impact by NVS is reduced to maintenance of shape change, if the photochemical treatment is applied to stretched tissue. It is devoid of cellular toxicity, but the photochemically introduced covalent bonds (formed via the photochemical reaction mechanisms noted above and described in [6]) increase the ECM density and stability leaving the treated tissue resistant to biological degradation, which decreases vascular inflammation (Figure 2).

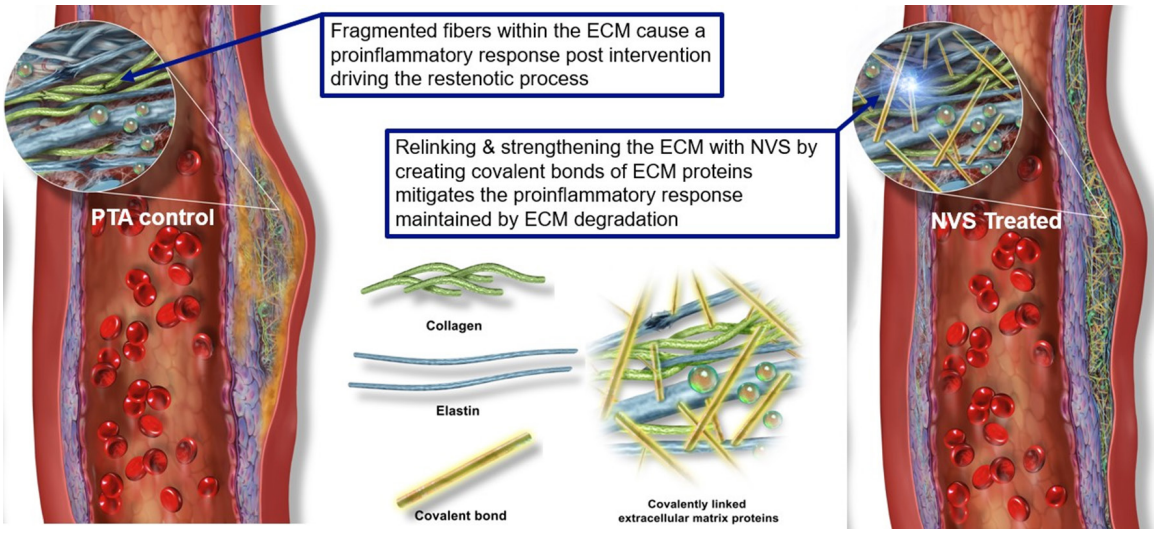

Figure 2: Artistic representation of the NVS effect on the arterial ECM. The illustration on the left-hand site depicts a magnified vessel wall with fragmented ECM fibers following percutaneous angioplasty (PTA). The artwork on the right-hand side illustrates a more stable lesion area following the angioplasty with NVS treatment. The middle of the figure captures the illustrations of the vessel wall elements with explanation.

Vascular applications

Cardiovascular tissue engineering requires unique interventions by which the treated tissue preserves its biological integrity. The simultaneous structural benefit needs to be coupled to retained functional properties (to compensate for loss of function mostly due to advanced atherosclerosis) or facilitate gain of function. Gain of function benefit is required in situations, such as arteriovenous fistula (AVF) creation and the need for subsequent AVF maturation. Maintaining vascular patency is the treatment goal in patients suffering from advanced atherosclerotic disease. Patent vessels prevent cardiac complications and limb loss. NVS is being developed as an endovascular treatment where 10-8-10 Dimer (described above) is coated on an angioplasty balloon to deliver the photochemical treatment to stenotic vessels.

The photochemical treatment contributes to preserving the angioplasty opened lumen by primarily targeting the ECM of the vessel wall. Applying the NVS treatment as an endovascular procedure and using limited light exposure by a light fiber imbedded inside the angioplasty balloon, which is coated by the photosensitive molecule, offers an easy one step procedure [9]. When the balloon is inflated, 10-8-10 Dimer diffuses into the vessel wall. After 60 seconds, a light fiber (integrated into the catheter) is illuminated initiating the photochemical reaction and subsequent covalent bond formation between proteins in the treated tissue while the balloon remains inflated. At completion of the procedure the light is turned off, balloon is deflated, and device is removed. The treatment aims to preserve the shape change of the lumen by the immediate opening of the vessel wall during the angioplasty procedure. The long-term biological outcome of the treatment is driven by the modified ECM and its regulatory role on subsequent cell proliferation and local inflammation.

Arteriovenous fistulas (AVFs) are the preferred form of vascular access for chronic hemodialysis of patients with end-stage renal disease. AVFs are created through the surgical or percutaneous anastomosis of a vein to the artery in the upper extremity. Successful AVF maturation can be facilitated with NVS therapy [10]. During AV fistula creation, a 10-8-10 Dimer coated balloon catheter is inserted into the vein. Inflation of the balloon dilates the vein. The balloon is inflated for a total of 2 minutes. The photochemical treatment takes

place during this time while the balloon is inflated. The NVS therapy works by promoting covalent bonding of extracellular matrix (ECM) protein fibers within the venous wall [10,11], which facilitates the arterialization process and aids AVF creation.

Role of ECM in remodeling

ECM proteins are signal generators and processors for the cellular components of the vascular wall [12]. Structural proteins connect with transmembrane proteins, such as integrins to elicit signal transduction and mediate cellular responses. Long-term outcome of interventions either on the arterial side or the venous side of the vascular system requires adaptation and remodeling of the vessel wall. This process is an active biological response, which is largely controlled by the quality of the ECM of the vessel wall. During the photochemical reaction by the NVS treatment, the newly formed covalent bonds of the ECM help to retain the treated shape of the vessel wall, which results in an immediate hemodynamic benefit. The treatment leaves the natural flexibility of the vessel wall intact and mitigates the physical activation of the vascular components caused by the physical stress from the sudden hemodynamic change, which plays a key role in the restenotic process. The photochemical linking of the ECM decreases and delays the degradation and fragmentation of the ECM fibers in the vessel wall, which contributes to decreased inflammation [9]. The NVS treatment does not affect cell proliferation directly, it modifies the ECM, which then contributes to a restorative remodeling of the vessel wall instead of an excessive hyperplastic response. Similarly, in case of AVF maturation, the treatment effect allows smooth muscle cell proliferation and thereby an effective arterialization of the vessel wall, but the modification of the ECM provides a control to this process resulting in a facilitated maturation with outward remodeling [10,11].

Conclusion

Photochemical tissue engineering carries great biomedical potential. The type of photochemical intervention (PDT, PTP, or NVS) is responsible for the extent of modification of the target tissue. For vascular indications the most promising modality is the so called NVS technology, which is safe for the cellular components of the vascular tissue, but exerts modification to the ECM through photochemically introduced covalent bond formation, leading to long-term benefits by supporting the necessary vascular remodeling.

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Conflict of Interest

Katalin Kauser and Kevin S Warner are employees of Alucent Biomedical Inc.

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