PROGRAMA DE DOCTORADO EN FÍSICA

TESIS DOCTORAL:

GENETICALLY ENGINEERED HYDROGELS BASED ON ELASTIN-LIKE RECOMBINAMERS FOR CARDIOVASCULAR APPLICATIONS

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1. ABSTRACT / RESUMEN

1.1. Abstract

Tissue engineering and regenerative medicine (TERM) is a prominent field of research that aims to repair or replace damaged tissues or organs that have lost their biological function due to lost, injury or disease. This multidisciplinary area of study is highly related to the Science of Biomaterials, which allows the development of scaffolds with essential features, such as biocompatibility and functionality, to overcome the current limitations that exist in the clinic. Nowadays, recombinant polypeptides arise as promising candidates due to their tunability at the genetic level, affording exquisite control over the final physico-chemical properties and bioactivities, thus, adapting to each specific biomedical application, considering the complexity of the human body. In particular, elastin-like recombinamers (ELRs) are genetically engineered polypeptides based on the repetition of the pentapeptide Val-Pro-Gly-X-Gly, a recurring sequence found in the hydrophobic domains of tropoelastin, where X can be any amino acid except L-Pro. Therefore, ELRs exhibit a reversible phase transition in aqueous environments above the so-called transition temperature (T_t), which is characterized by a change in the protein conformation. This thermal response enables a cost-effective purification through heating and cooling cycles, a process known as inverse transition cycling (ITC). Furthermore, their recombinant nature allows the inclusion of specific motifs, such as cell adhesion and protease-sensitive sequences, and biologically active molecules such as growth factors. Interestingly, they can be chemically modified to obtain covalently cross-linked hydrogels through the fast, orthogonal and cytocompatible "click chemistry" reactions.

The first chapter of this thesis is dedicated to the spatiotemporal control of angiogenesis, which has been proven essential for the correct integration and

long-term stability of the implant. To this end, we designed a coaxial binary elastin-like recombinamer (ELR) tubular construct that displays fast and slow protease-sensitive sequences towards the urokinase plasminogen activator protease on its inner and outer part respectively. The ELRs further included the universal cell adhesion domain (RGD) and a VEGF-mimetic tethered peptide (QK) to induce angiogenesis. *In vitro* studies evidenced the effect of the QK peptide on endothelial cell extension and anastomosis. The subcutaneous implantation of the three-dimensional (3D) model constructs in mice showed a guided cell infiltration and capillary formation in the pre-designed spatiotemporal arrangement of the construct, with a higher rate for the fast ELR hydrogels and in the presence of the QK peptide. Thus, an inside-to-outside angiogenesis pattern, in contrary to the natural evolution of host-cell colonization was confirmed. Furthermore, the ELR hydrogels induced a mild macrophage response that resolved over time, supporting the potential adaptability and integration of the resorbable scaffold within the host tissue. These results set the basis for the application of this type of scaffolds in regenerative medicine applications where spatiotemporally controlled vascularization plays an important role.

The second chapter of this thesis study the preferential guidance of angiogenesis and an often-overlooked neurogenesis in a spatiotemporal manner, offering a non-protease-sensitive, as well as slow-resorbable ELR version scaffolds, intended for each specific application. In particular, we designed a 3D model elastin-like recombinamer (ELR) scaffold comprising two internal cylinders, with the pro-angiogenic peptide (QK) in one of them, and the neuronal cell adhesive peptide (IKVAV) in the vicinal one, both covalently tethered. In addition, these cylinders contain proteolytic sequences with fast cleavage kinetics towards the urokinase plasminogen activator enzyme and RGD cell adhesive domains. On the other hand, the outer part

displays a slow-resorbable or non-protease-sensitive ELR hydrogel. *In vitro* studies demonstrated the effect of IKVAV epitope on neurite extension and the effect of QK epitope on human umbilical vein endothelial cells (HUVECs) anastomosis. The subcutaneous implantation of the 3D model ELR constructs in mice showed a guided cell infiltration accompanied by preferential angiogenesis or innervation on the respective QK and IKVAV containing cylinders, enabling a highly defined unidirectional pattern, with a faster integration within the host tissue for the slow-resorbable scaffold. These results widen the applications of ELR scaffolds for tissue engineering and regenerative medicine strategies aiming to mimicry the native three-dimensional architecture of vascular and nervous native systems.

The third chapter of this thesis describes the development of a ready-to-use bi-leaflet transcatheter venous valve for the treatment of chronic venous insufficiency (CVI), a leading worldwide vascular disease. For this purpose, we combined (i) elastin-like recombinamers, (ii) a textile mesh reinforcement and (iii) a bioabsorbable magnesium stent. Burst strength analysis demonstrated mechanical properties suitable for vascular pressures, whereas equibiaxial analysis confirmed the anisotropic performance equivalent to the native saphenous vein valves. *In vitro* studies identified the non-thrombogenic, minimal hemolytic and self-endothelialization properties endowed by the ELR hydrogel. The hydrodynamic testing under pulsatile conditions revealed minimal regurgitation (< 10%) and pressure drop (< 5 mmHg) in accordance with values stated for functional venous valves, and no stagnation points. Furthermore, *in vitro* simulated transcatheter delivery showed the ability to withstand the implantation procedure. These results set the basis for the development of bioresorbable transcatheter venous valve substitutes with great potential for CVI treatment.

In summary, the thesis presented herein provide new insights in the design and development of novel ELR-forming hydrogels to be used in tissue engineering and regenerative medicine applications. Specifically, it focuses on the combination of different bioactive domains decorated in ELRs via genetic engineering or chemical attachment, which selectively located in fabricated 3D model hydrogels can serve for pre-programmed spatiotemporal cell fate modulation *in vivo*. In addition, it exploits the hemocompatible and self-endothelialization properties of ELR scaffolds for their application in a ready-to-use bi-leaflet transcatheter bioabsorbable venous valve prototype for CVI treatment.

1.2. Resumen

La ingeniería de tejidos y la medicina regenerativa (TERM) es un campo de investigación destacado que tiene como objetivo reparar o reemplazar tejidos u órganos dañados que han perdido su función biológica debido a lesión o enfermedad. Este área de estudio multidisciplinar está muy relacionada con la Ciencia de los Biomateriales, que permite el desarrollo de andamios con características esenciales como la biocompatibilidad y la funcionalidad, para superar las limitaciones que existen actualmente en clínica. En la actualidad, los polipéptidos recombinantes surgen como candidatos prometedores debido a su capacidad de diseño a nivel genético, lo que permite un control exquisito sobre las propiedades físico-químicas y bioactividades finales. Pudiendo de este modo adaptarse a cada aplicación biomédica concreta, considerando la complejidad del cuerpo humano. En particular, los recombinámeros de tipo elastina (ELR) son polipéptidos modificados genéticamente basados en la repetición del pentapéptido Val-Pro-Gly-X-Gly, una secuencia recurrente que se encuentra en los dominios hidrófobos de la tropoelastina, donde X puede ser cualquier aminoácido excepto prolina. Por lo tanto, los ELR presentan una

transición de fase reversible en entornos acuosos por encima de la denominada temperatura de transición (Tt), que se caracteriza por un cambio en la conformación de la proteína. Esta respuesta térmica permite una purificación rentable a través de ciclos de calentamiento y enfriamiento, un proceso conocido como ciclo de transición inversa (ITC). Además, su naturaleza recombinante permite la inclusión de epítopos específicos, como por ejemplo secuencias de adhesión celular, secuencias proteolíticas o moléculas biológicamente activas como factores de crecimiento. Curiosamente, estos ELRs se pueden modificar químicamente para obtener hidrogeles entrecruzados covalentemente a través de reacciones rápidas, ortogonales y citocompatibles de "química de clic".

El primer capítulo de esta tesis está dedicado al control espacio-temporal de la angiogénesis, la cual ha demostrado ser fundamental para la correcta integración y estabilidad a largo plazo del implante. Para ello, diseñamos un modelo tridimensional (3D) que consiste en una construcción binaria coaxial de hidrogeles de recombinámeros de elastina (ELR), que lleva secuencias proteolíticas con cinética de escisión rápida y lenta sensibles frente a la proteasa del activador del plasminógeno tipo uroquinasa en su parte interna y externa respectivamente, y un péptido mimético de VEGF (QK) anclado para inducir la angiogénesis. Los estudios *in vitro* evidenciaron el efecto del péptido QK sobre la extensión y anastomosis de las células endoteliales. La implantación subcutánea del modelo 3D en ratones mostró una infiltración celular guiada y la formación de capilares en la disposición espacio-temporal prediseñada del andamio, con una velocidad más alta para los hidrogeles ELR con secuencias sensibles a proteasas rápidas y en presencia del péptido QK. Así, se confirmó un patrón de infiltración de adentro hacia afuera, contrario a la evolución natural de la colonización. Además, los hidrogeles ELR indujeron una respuesta leve de macrófagos que se resolvió con el tiempo, lo que respalda

la adaptabilidad e integración potencial de estos andamios reabsorbibles dentro del tejido hospedador. Estos resultados sientan las bases para la aplicación de este tipo de andamios en aplicaciones de medicina regenerativa donde la vascularización controlada de manera espacio-temporal juega un papel importante.

El segundo capítulo de esta tesis estudia la orientación preferencial de la angiogénesis y la neurogénesis (a menudo pasada por alto), de una manera espacio-temporal, ofreciendo una versión de andamios de ELR no sensibles a proteasas, así como una de lenta reabsorción, pensada para cada aplicación especifica. En particular, diseñamos un modelo 3D que contiene dos cilindros basado en recombinámeros de tipo elastina (ELR), que comprende el péptido pro-angiogénico (QK) en un cilindro, y el péptido adhesivo a células neuronales (IKVAV) en el cilindro contiguo, ambos anclados covalentemente. Además, estos túbulos contienen secuencias proteolíticas con cinética de escisión rápida frente a la enzima del activador del plasminógeno tipo uroquinasa, así como dominios de adhesión celular RGD. Por otro lado, la parte exterior presenta un hidrogel de ELR de lenta reabsorción o uno no sensible a proteasas. Los estudios *in vitro* demostraron el efecto del epítopo IKVAV sobre la extensión de axones o dendritas y el efecto del epítopo QK sobre la anastomosis de HUVECs. La implementación subcutánea de los modelos 3D de ELR en ratones demostró una infiltración celular guiada acompañada de angiogénesis o inervación preferencial en los respectivos cilindros conteniendo QK o IKVAV. Además, la parte exterior no sensible a proteasas o de reabsorción lenta de los andamios permite el desarrollo de patrón unidireccional altamente definido de infiltración celular, con una integración más rápida dentro del tejido hospedador en el caso del andamio con reabsorción lenta. Estos resultados amplían las aplicaciones de los andamios de los ELR en la ingeniería de tejidos y medicina regenerativa cuyo

objetivo es el de imitar la arquitectura tridimensional nativa de los sistemas vasculares y nerviosos.

El tercer capítulo de esta tesis describe el desarrollo de una válvula venosa transcatéter biválvula lista para usar sin preacondicionamiento con células *in vitro* para el tratamiento de la insuficiencia venosa crónica (IVC), una de las principales enfermedades vasculares a nivel mundial. Para este propósito, combinamos (i) recombinámeros de tipo elastina, (ii) un refuerzo de malla textil y (iii) un stent de magnesio bioabsorbible. El análisis de resistencia al rotura demostró propiedades mecánicas adecuadas para las presiones vasculares, mientras que el análisis equibiaxial confirmó el rendimiento anisotrópico equivalente a las válvulas de la vena safena nativa. Los estudios *in vitro* identificaron las propiedades no trombogénicas, de hemólisis mínima y de autoendotelización que otorga el hidrogel de elastina recombinante. Las pruebas hidrodinámicas en condiciones pulsátiles revelaron una regurgitación mínima (< 10 %) y una caída de presión (< 5 mmHg) de acuerdo con los valores establecidos para válvulas venosas funcionales y sin puntos de estancamiento. Además, el suministro transcatéter simulado *in vitro* demostró la capacidad de la válvula de soportar el procedimiento de implantación. Estos resultados sientan las bases para el desarrollo de sustitutos de válvulas venosas transcatéter bioreabsorbibles con un gran potencial para el tratamiento de la IVC.

En resumen, la tesis presentada en este documento proporciona nuevos conocimientos en el diseño y desarrollo de nuevos hidrogeles basados en recombinámeros de tipo elastina (ELR) para su uso en la ingeniería de tejidos y en aplicaciones de medicina regenerativa. Específicamente, se enfoca en la combinación de diferentes dominios bioactivos decorados en los ELR a través de ingeniería genética o unión química, que ubicados selectivamente en

modelos 3D pueden servir para la modulación espaciotemporal preprogramada del comportamiento celular *in vivo*. Además, aprovecha las propiedades hemocompatibles y de autoendotelización de los hidrogeles de ELR para su aplicación en un prototipo de válvula venosa bioabsorbible transcateterizable lista para usar sin preacondicionamiento *in vitro* con células para el tratamiento de la insuficiencia venosa crónica (IVC).

2. INTRODUCTION

Introduction

Tissue engineering and regenerative medicine

Almost since the start of the human being, the multidisciplinary practice of medicine has been evolving trying to solve all the different illness and diseases that affect the inner nature of the human body. The improvement of intelligence and later of science and technology increasingly provided solutions for the repair or replacement of damaged tissues and organs, enlarging what we know today as Regenerative Medicine [1–3]. To this end, varieties of materials have been designated as possible substitutes. Firstly wood, ceramics and metals were used to this purpose, evolving to more sophisticated materials that closely imitate the complexity of the human body. In this sense, synthetic polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) or poly(lactide-co-glycolide) (PLGA), were used as scaffolds [4–6]. These biomaterials can be fabricated reproducibly and further allow tailorable architectures, mechanical properties, and degradation rates [7,8]. However, synthetic polymers are generally characterized by a hydrophobic nature and the lack of biologic recognition, which increases the risk of rejection [9,10]. On the other hand, appear the biomaterials obtained from a natural source, such as collagen, silk, fibronectin, laminin, elastin or chitosan [11,12]. This class of materials more effectively emulate the biological properties of the native extracellular matrix (ECM), since they provide cellular signaling (cell migration, proliferation, adhesion and differentiation), a biodegradable microenvironment and minimize the inflammatory host-response favoring the integration within the host tissue [13,14]. However, despite the advantages mention above, there is limited supply of these biomaterials, reproducibility between batches presents a challenge, and the transfer of pathogens is possible [11,15]. Among biomaterials, the protein-based exhibit excellent properties,

such as high biocompatibility and tailorable functionalization, highlighting between them the elastin-based, which further incorporate the exceptional resilience and elasticity displayed by many tissues and organs such as lungs, blood vessels, ligaments or skin [16,17].

Elastin fibers

Elastin fibers are insoluble, long-lasting proteins, which are found in elastic tissues and organs and provides structural integrity, elasticity, as well as biological activity [18,19]. The fabrication of elastic fibers in the body consists of the association of its soluble precursor tropoelastin that is covalently cross-linked by the lysyl oxidase (LOX) enzymes, in a process called elastogenesis [20–22]. Tropoelastin is an intrinsically disordered protein (IDP) that can reversibly stretch its length up to eight times, which confers the great elasticity and mechanical resilience exhibited by elastin [23]. Despite the importance of elastin fibers in tissues and organs, reduced tropoelastin expression and elastic fiber assembly occurs in adults leading to a detrimental in tissue repair [19]. Highly repeated motifs found in tropoelastin have been shown to be responsible for endowing the outstanding properties of elastin fibers, including the Val-Pro-Gly-Val-Gly (VPGVG) pentapeptide described by Gray et al. [24]. In that sense, the development of recombinant DNA technology over the last decades allows the fabrication of polypeptides (biomaterials) with repeated elastin-like sequences, which are known nowadays as elastin-like recombinamers (ELRs) [25–27].

Elastin-like recombinamer (ELR) biomaterials

Elastin-like recombinamers (ELRs) are genetically engineered polypeptides fabricated by recombinant synthesis and usually expressed in *Escherichia coli* bacteria, which are based on the repetition of the Val-Pro-Gly-Xaa-Gly (VPGXG) pentapeptide, where X can be any amino acid except proline

[25,28]. Due to its composition, ELRs exhibit a reversible lower critical solution temperature (LCST) in aqueous solution [29]. Thus, below a characteristic temperature, known as transition temperature (T_t) the ELR molecules are hydrated surrounded by clathrate water structures. However, above this T_t the ELR molecules folds hydrophobically and self-associate forming a separate state. This reversible coacervation, termed as inverse temperature transition (ITT), depends on the hydrophobicity of the fourth amino acid, the ELR concentration, the molecular weight, the pH, the pressure, as well as the presence and concentration of other molecules and ions [30]. Interestingly, this thermos-responsiveness behavior exhibited by the ELRs can be exploited for their simple purification from the debris after *E. coli* lysis, by a process called inverse transition cycling (ITC), which is based on cooling and heating cycles [31]. In addition, their recombinant nature allow (i) the precise control over their physicochemical properties and polypeptide composition, (ii) their chemical modification with bioactive or crosslinking domains and (iii) their monodisperse bioproduction, which makes them a novel source for the development of tailorable biomaterials with advanced functionalities for a myriad of tissue engineering and regenerative medicine applications [25,32,33].

Elastin-like recombinamer hydrogels

Hydrogels are hydrated highly porous 3D scaffolds very promising in TE since they provide similar mechanical and biochemical properties to that experienced by tissues *in vivo*. In addition to the basic support, they can mediate specific cellular responses as cell migration and differentiation, supporting integration in the host tissue and finally matrix synthesis [34]. Interestingly, ELR hydrogels can be obtained via physical or chemical crosslinking. Thus, physical hydrogels have been created by hydrogen bond-,

hydrophobic- and electrostatic interactions, as well as supramolecular rearrangements such as β-sheets [35]. On the other hand, chemical hydrogels have also been obtained through different approaches, highlighting between them the use of the strain-promoted [3 + 2] azide-alkyne cycloaddition (SPAAC) [36,37]. This catalyst-free click chemistry reaction presents several advantages as provides high yields, is fast and cytocompatible so can be performed in aqueous solution under mild physiological environments with no release of toxic by-products [38]. To this end, ELRs are decorated with either azide or cyclooctyne crosslinkable groups taking advantage of the ε-amino group present in the in the side chains of lysine amino acids, which upon mixture reacts orthogonally to form an irreversible covalent bond between the ELR molecules leading to a stable hydrogel [39]. In addition, the crosslinking density can be modulated by varying the density of azide and cyclooctyne groups, allowing the control of the mechanical properties and network pore size. This chemical modification of ELRs through the lysine groups, as well as, by the click chemistry reaction, further opens the possibility to tailor the ELR chains by including different bioactive compounds, suggesting these ELR-based hydrogels as one of the most promising candidates for a wide variety of tissue engineering and regenerative medicine applications [40].

Advanced ELRs hydrogels for spatiotemporal guided angiogenesis

The spatiotemporal promotion of angiogenesis is a major topic of research in tissue engineering, which is key for the repair of many tissues and organs, to finally create a properly integrated functional capillary network that replicates the native structure [41–46]. Potential solutions comprise the use of degradable hydrogels encapsulating growth factors in pre-determined spatial orientations [44,47]. However, this usually lead to vascular malformations, immature networks or tumorigenesis due to the initial burst release of the

factors [48–51]. Other approaches that have shown promising results in the orientated development of vasculature include hydrogels with different spatial protease-sensitive kinetics (cellular migration rate), and the use of covalently tethered angiogenic growth factors that impede the burst release [39,47–50,52]. The recombinant and tunable nature of ELR hydrogels allow the combination of all of the aforementioned properties within the same 3D scaffold, thus presenting them as promising candidates for the spatiotemporal guidance of angiogenesis within TE implants.

Advanced ELRs hydrogels for spatiotemporal guided neurogenesis

The development of spatiotemporal neurogenesis within hydrogels has always been either an overlooked, or a highly complex objective pursued in tissue engineering [53,54]. This process consists of proteases release, axonal growth and proliferation of Schwann cells, to finally create a replica of the neural networks that provide the lost sensory and motor functions and ensure the successful integration of the scaffold within the host tissue [55–57]. The encapsulation of growth factors have shown promising results to this end [58]. However, the complex control over gradient distribution and release pharmacokinetics has been related to dose-related adverse effects and cytotoxic effects [59–61]. Complementarily, the spatial exposition of laminin-derived peptides in degradable hydrogels has demonstrated the guidance of neural attachment and outgrowth, although light or temperature gradients needed to be applied selectively [62–64]. ELRs hydrogels, as advanced recombinant and tunable biomaterials allow the spatiotemporal location of specific neuronal cell adhesion domains, and protease-sensitive sequences (cellular infiltration rate), thus holding great potential for the spatiotemporal guidance of neurogenesis and their use in complex scaffolds toward neural regeneration.

Advanced ELRs hydrogels for the development of off-the-shelf venous valves

Lower extremity chronic venous insufficiency (CVI) is a major health issue worldwide affecting between 5 and 33% of the adult population that results in painful swelling, venous ulcers, varicose veins, thrombus formation, edema, and skin ulcerations [65–72]. Current solutions are scarce comprising between them: (i) classic therapies, such as anticoagulants and compression devices, (ii) autologous venous valve replacement, (iii) allo and xenografts, and (iv) polymeric and metallic materials such as pellethane or platinum [73–78]. However, these solutions target just the symptoms, present limited availability and patient comorbidity or usually result in thrombosis and immunogenic rejection, with occlusion, fibrosis and hyperplasia, respectively, leading to no single venous valve prosthesis available in clinics [73–78]. This scenario prompts the development of relevant venous valve replacements that should (i) present adequate mechanical properties (ii) avoid any pre-condition *in vitro* phase (to be an off-the-shelf substitute), (iii) avoid immunogenic rejection, (iv) allow host cell endothelialization, (v) be antithrombogenic, (vi) be hemocompatible, (vii) tolerate the crimping procedure upon transcatheter delivery, and (viii) display cell adhesive properties and protease-sensitiveness (to improve integration) [39,79–87]. Despite ELR hydrogels have been used for a number of vascular graft devices [84,86–88], no single ELR-based venous valve model has been developed to date in the literature, suggesting them as powerful candidates to meet such demands and develop a venous valve substitute in combination with a textile reinforcement and a magnesium degradable stent toward the goal of CVI treatment.

3. OBJECTIVES

Objectives

The main objective of this thesis is to develop multifunctional ELR-based biomaterials capable to provide advances in the field of tissue engineering and regenerative medicine. To this end, we take advantage of the versatility of ELRs for the development of different materials that comprise diverse bioactive domains, included via genetic engineering or chemical attachment, to improve the biological responses at the application level.

The first study aims to design a 3D model ELR construct with a pre-programmed spatiotemporal orientation for cell colonization and angiogenesis. Click crosslinkable ELRs bearing cell-adhesive, pro-angiogenic and protease-sensitive domains will be employed for the fabrication of the constructs. The effect of the pro-angiogenic domain over HUVEC morphogenesis will be evaluated *in vitro*, whereas subcutaneous implantation in mice will be used to analyze the macrophage response and the spatiotemporal direction of angiogenesis.

The objective of the second study is to develop a 3D model ELR construct displaying two internal cylinders for the preferential guidance of angiogenesis and neurogenesis in space and time. Domains differing in terms of pro-angiogenic and neuronal cell adhesion properties will provide each cylinder bioactivity, whereas protease-sensitive domains will serve for creating a defined oriented cell infiltration. The effect of neuronal cell adhesive domains over neurite extension will be studied *in vitro*, whereas the spatiotemporal evolution of neurogenesis and angiogenesis will be evaluated by *in vivo* studies.

In the third study we aim at the development of a ready-to-use bi-leaflet transcatheter venous valve prosthesis for chronic venous insufficiency (CVI) treatment. Elastin-like recombinamers will provide a suitable

microenvironment for cell infiltration and tissue generation *in situ*, a textile mesh will provide mechanical reinforcement and a bioabsorbable magnesium stent the structural support. The anisotropic and mechanical behavior will be analyzed by equibiaxial and burst strength studies, whereas hemocompatibility, self-endothelialization, hydrodynamic performance and transcatheter delivery will be evaluated *in vitro*.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1 Chemical reagents

Table of chemical reagents employed in the thesis and commercial houses.

Reagent (abbreviation)	Comercial House
ELR modifications	
2-azidoethyl (2,5-dioxopyrrolidin-1-yl) carbonate	GalChimia
(1R,8S,9S)-bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate	GalChimia
H-Cys[(3-succinimido-propionyl)-B-Ala-DBCO]-Gln-Ala-Ala-Ser-Ile-Lys-Val-Ala- Val-OH trifluoroacetate salt	Bachem
H-Cys[(3-succinimido-propionyl)-B-Ala-DBCO]-Lys-Leu-Thr-Trp-Gln-Glu-Leu-Tyr- Gln-Leu-Lys-Tyr-Lys-Gly-Ile-OH trifluoroacetate salt	Bachem
NHS-Cyanine5 (Cy5)	Jena Bioscience
Dimethylformamide (DMF)	Sigma-Aldrich
Dimethylsulfoxide (DMSO)	Sigma-Aldrich
Diethyl ether	Sigma-Aldrich

Dimethyl sulfoxide-D6	Sigma-Aldrich
Cell culture	
Phosphate buffer saline pH 7.2 (PBS 1X)	Gibco
EBM-2 basal medium (CC-3156)	Lonza
EGM-2 SingleQuots (CC-4176)	Lonza
Fetal bovine serum (FBS)	Lonza
Penicillin	Lonza
Streptomycin	Lonza
Soluble human VEGF recombinant protein ($VEGF_{165}$)	Lonza
F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) (ATCC® 30-2004™)	ATCC
Horse serum, New Zealand origin	Gibco
Trypan blue	Invitrogen
Endothelial cell growth medium (EGM)	PromoCell
Basic Fibroblast growth factor	PromoCell
Insulin-like growth factor	PromoCell
Ascorbic acid	PromoCell
Hydrocortisone	PromoCell
Fetal calf serum FCS	PromoCell
Trypsin/EDTA 0.05%	Gibco

Histology and immunohistochemistry	
Paraformaldehyde (PFA)	Sigma-Aldrich
Triton™ X-100	Sigma-Aldrich
4 ',6-diamidino-2-fenilindol (DAPI)	Thermo Fisher/Roth
Phalloidin-Alexa Fluor 488	Thermo Fisher
Optimum cutting temperature (OCT)	Sigma Aldrich
Aqueous mounting medium	VWR
Aqueous fluoroshield mounting medium with DAPI	Abcam, ab104139
Hematoxylin	Sigma Aldrich
Eosin	Sigma Aldrich
Acetone	Sigma Aldrich
10% Normal goat serum	Life Technologies/Dako
Rabbit anti-CD31 primary antibody	Abcam, ab28364
Rabbit anti-CCR7 primary antibody	Abcam, ab32527
Rabbit anti-CD163 primary antibody	Abcam, ab182422
Goat anti-rabbit IgG H&L Alexa Fluor 488 secondary antibody	Abcam, ab150077
Neuronal marker anti-beta III tubulin primary antibody	Abcam, ab18207
Rat anti-mouse CD31 primary antibody	BD Pharmingen, 553370
Anti-CD31 mouse primary antibody	Sigma Aldrich

Goat anti-rat IgG (H+L) Alexa Fluor 546 secondary antibody	Invitrogen, A-11081
Goat anti-mouse IgG H&L Alexa Fluor 594 secondary antibody	Invitrogen, A11005
Xylol	Sigma-Aldrich
Bovine serum albumin (BSA)	Sigma-Aldrich
Rhodamine-Phalloidin R415	Invitrogen
Ethanol (EtOH)	Panreac
Glutaraldehyde	Roth
ELR bioproduction	
Escherichia coli XL-1 Blue competent cells	Agilent
Escherichia coli XL-1 Blue subcloning	Agilent
Escherichia coli BLR	ThermoFisher Scientific
Bacterial culture medium (Luria Bertani, LB)	Conda
Auto-induction culture medium “Terrific” (TB)	Foremedium
Antifoam 204	Sigma-Aldrich
Agar	BD
Glycerol	Fisher Scientific
Glycine	Sigma-Aldrich

Glucose	Panreac
Restriction enzymes (*Sap*I, *Ear*I, *Eco*RI, *Dpn*I) and other enzymes (T4 DNA ligase, FastAP, SAP)	Fisher Scientific
Ampicillin	Apollo Scientific
Kanamycin	Apollo Scientific
4-(2-Aminoethyl) benzenesulphonyl fluoride hydrochloride (AEBSF)	Apollo Scientific
Phenylmethylsulfonyl fluoride (PMSF)	Apollo Scientific
Leupeptin	Apollo Scientific
Pepstatin A	Apollo Scientific
Ammonium persulphate	Fisher Scientific
Sodium chloride (NaCl)	Fisher Scientific
Agarose metaphor	Cambrex
Agarose seakem	Cambrex
Ultrapure water (MilliQ)	Millipore
Simply safe DNA stain	Eurx
Plasmid purification Kit “NucleoSpin Plasmid”	Macherey-Nagel
DNA extraction kit in agarose gels “PureLink Quick Gel Extraction”	Invitrogen
DNA marker 1kb Plus Ladder	Invitrogen

Acrylamide/Bis-acrylamide	Amresco
Sodium hydroxide (NaOH)	Fisher Scientific
Chlorhydric acid (HCl)	Fisher Scientific
Tris(hydroxymethyl)aminomethane (Tris)	Sigma-Aldrich
Tetramethylethylenediamine (TEMED)	Sigma-Aldrich
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich
β-mercaptoethanol	Sigma-Aldrich
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich
Protein marker “Pierce Unstained”	Fisher Scientific
Copper chloride	Sigma-Aldrich
n-butanol (BuOH)	Sigma-Aldrich
Tetrakis(hydroxymethyl)phosphonium chloride	Fisher Scientific
Cupper chloride ($CuCl_2$)	Sigma-Aldrich
E-64	Apollo Scientific
Coomassie brillant-blue R-250	Sigma-Aldrich
Isopropanol	Invitrogen
Methanol	Sigma-Aldrich
Others	
Isoflurane	Sigma Aldrich
2-methylbutane	Sigma Aldrich

Heparin sodium salt	Sigma-Aldrich
Thrombin from bovine plasma T4648, 40 U/mL	Sigma Aldrich
Tris-buffered saline (TBS) pH 7,4	Sigma Aldrich
Calcium chloride dihydrate	Sigma Aldrich
Fibrinogen from human plasma 341576	Calbiochem
Sodium citrate tribasic dihydrate 0.3%	Sigma Aldrich
Silicone elastomer 184	Sylgard®
Silicone elastomer curing agent 184	Sylgard®

4.1.2 Cell lines

Human umbilical vein endothelial cells (HUVECs, PCS-100-010, ATCC®). Cells between passages 3 and 5 were used. C6 glial cells (CCL-107™, ATCC®). Cells between passages 2 and 4 were used. Human umbilical vein endothelial primary cells (HUVECs) provided by the AME institute (department of Gynaecology of the University Hospital Aachen) from three different donors. Cells at passage 3 were used.

4.1.3 Animal model

Female 3-month Swiss CDR-1 mice provided by the University of Valladolid animal facility.

4.1.4 Elastin like recombinamers (ELRs)

List of ELRs employed in this thesis

ELR	Amino acid Sequence	MW (Da)
RGD	MGSSHHHHHHSSGLVPRGSHMESLLP ([(VPGIG)$_2$(VPGKG)(VPGIG)$_2$]$_2$AVTG**RGD**SPASS [(VPGIG)$_2$(VPGKG)(VPGIG)$_2$]$_2$)$_6$ V	60650
VKV	MESLLPVGVPGVG[VPGKG(VPGVG)$_5$]$_{23}$ VPGKG(VPGVG)$_3$ VPGV	60451
DRIR	MESLLPV([(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_2$YAVTG**D RIR**SASPASSA[(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_2$V)$_4$	42069
GTAR	MESLLPV([(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_2$YAVTG**G TAR**SASPASSA[(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_2$V)$_4$	41448

4.1.5 Equipment and other materials

48 multiwell plate (Corning), 96 well plates (VWR®), millicell EZ slice 8-well (Thermo Scientific), micropipettes and tips (Gilson), needles and syringes (BD), glass beakers, ballons, Erlenmeyer flasks and pipettes (Scharlab), centrifuge tubes and cell culture flasks (Fisher Scientific), microcentrifuge tubes (Eppendorf), NMR tubes (Wildmad), autoclave (Selecta Autotester E-75), inverted Nikon Eclipse Ti-E epifluorescence microscope coupled to a DS-2MBWc camera with NIS-Elements AR software (Nikon Corporation), Leica TCS SP8 LIGHTNING confocal microscope (Leica Microsystems), Inverted Zeiss LSM 710 confocal microscope with Zen (blue edition) software (©Carl Zeiss Microscopy GmbH), sutures (ProleneTM, EP8703, 7-0), Cryostat (Shandon Cryotome E Cryostat, Thermo scientific), textile mesh (ITA), GORE-TEX®, magnesium degradable stent (MeoTec), mixing nozzle (Automix-Tips, DMG), spectrophotometer (Tecan infinite 200), VHX-5000 digital microscope, camera (Olympus Stylus SH-1), biaxial tester

(Zwick Roell Biaxial Tester) with software (TestXpert II V3.71), peristaltic pump (IPC Ismatec, IDEX Health & Science GmbH), pressure sensor (Jumo Midas pressure transmitter; JUMO GmbH & Co. KG), custom made software (LabVIEW, National Instruments), pulsatile pump (702-6882, RS components), silicone rubber (Ismatec), flow sensor (SonoTTTM Flowcomputer, em-tec GmbH), expandable balloon (PTA dilation catheter, Atlas Gold), silicon tube (Sigma Aldrich) and 3D printer (Formlabs Form 2).

4.2. Methods

4.2.1. Synthesis of elastin-like recombinamers (ELRs)

ELRs were designed, bioproduced and purified as described elsewhere. Briefly, genes encoding the ELRs amino acid sequences (NZYTECH) were cloned in the plasmid pDriveAll, using *E. coli* XL1 blue competent cells (Agilent), and "seamless" fused by the EarI and SapI restriction enzymes (Fermentas). The final gene was extracted from the cloning plasmid and cloned in expression plasmid pET7RARE, resulting from different modifications on the commercial vector pET-25b (+) (Novagen). Expression BLR (DE3) *E. coli* strains (Novagen) were transformed with the plasmid and cultured in a 15 L bioreactor (Applikon Biotechnology) for 16 h under controlled conditions of pH, temperature and agitation. Bacterial cells were mechanically disrupted (TS 0.75KW, Constant Systems) and the ELRs were purified by inverse transition cycling (ITC) and centrifugation steps. Pure ELRs were dialyzed (MilliQ ultrapure water), filtrated (0.22 µm, Nalgene) and freeze-dried (FreeZone 1, LABCONCO) for long term preservation.

4.2.2. Chemical modification of ELRs

Detailed chemical reactions for the preparation of the ELR-modified versions.

VKV-azide: 2-azidoethyl (2,5-dioxopyrrolidin-1-yl) carbonate (135.4 mg, 18 eq.) was added to a solution of the ELR VKV in DMF (2000 mg in 100 mL). The resulting mixture was stirred at room temperature (r.t.) for 60h. After this time, diethyl ether (300mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 mL) by centrifugation (12500 G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100 mL), dialyzed against ultrapure water at 4ºC (3 x 25 L), the pH of the solution was adjusted to 7, filtered (0.22 µm) and freeze-dryed to yield a white recombinamer with the 60% of the lysine groups modified with azide (VKV-N_3).

VKV-cyclooctyne: (1R,8S,9S)-bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (154.6 mg, 16 eq.) was added to a solution of the ELR VKV in DMF (2000 mg in 100 mL). The resulting mixture was stirred at room temperature (r.t.) for 60h. After this time, diethyl ether (300mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 mL) by centrifugation (12500 G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100 mL), dialyzed against ultrapure water at 4ºC (3 x 25 L), the pH of the solution was adjusted to 7, filtered (0.22 µm) and freeze-dryed to yield a white recombinamer with the 60% of the lysine groups modified with cyclooctyne (VKV-cyclooctyne).

VKV-cyclooctyne-Cy5: a solution of NHS-Cyanine5 (0,0177 mmol, 1eq) in DMF was added to a solution of VKV-cyclooctyne (0,0177 mmol, 1100 mg) in DMF (5mL) and the resulting mixture was stirred at r.t. overnight. After this time, diethyl ether (30mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 20 ml) with centrifugation (12500G, 10 min), dried under reduced pressure,

redissolved in cold ultrapure water at 4ºC and dialyzed against MQ water (3 x 25 L). Then the pH of the solution was adjusted to 7, the product was filtered (0.22 μm) and lyophilized to yield a blue recombinamer with 1eq of the lysine groups modified with Cy5 (VKV-cyclooctyne-Cy5).

RGD-azide: 2-azidoethyl (2,5-dioxopyrrolidin-1-yl) carbonate (135.4 mg, 18 eq.) was added to a solution of the ELR RGD in DMF (2000 mg in 100 mL). The resulting mixture was stirred at room temperature (r.t.) for 60h. After this time, diethyl ether (300mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 mL) by centrifugation (12500 G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100 mL), dialyzed against ultrapure water at 4ºC (3 x 25 L), the pH of the solution was adjusted to 7, filtered (0.22 μm) and freeze-dryed to yield a white recombinamer with the 70% of the lysine groups modified with azide (RGD-N_3).

RGD-azide-Cy5: a solution of NHS-Cyanine5 (0,0177 mmol, 1eq) in DMF was added to a solution of RGD-azide (0,0177 mmol) in DMF (5mL) and the resulting mixture was stirred at r.t. overnight. After this time, diethyl ether (30mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 20 ml) with centrifugation (12500G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water at 4ºC and dialyzed against MQ water (3 x 25 L). Then the pH of the solution was adjusted to 7, the product was filtered (0.22 μm) and lyophilized to yield a blue recombinamer with 1eq of the lysine groups modified with Cy5 (RGD-azide-Cy5).

RGD-azide-QK: a solution of H- Cys [(3- succinimido- propionyl)- β-Ala- DBCO]- Lys-Leu-Thr-Trp-Gln-Glu-Leu-Tyr-Gln-Leu-Lys-Tyr-Lys-Gly-Ile-OH trifluoroacetate salt (0.01336 mmol, 1,2 eq) in DMF (0,5mL) was

added to a solution of the RGD-azida in DMF (0.01114 mmol in 15 ml). The resulting mixture was stirred at room temperature (r.t.) for 8h. After this time, diethyl ether was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 ml) with centrifugation (12500G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100mL), dialyzed against ultrapure water at 4ºC (3 x 25 L). Then the pH solution was measured and adjusted to 7, filtered (0.22 µm) and lyophilized to yield a white recombinamer with 0.5 eq of the zide groups modified with QK (RGD-azide-QK).

RGD-azide-QK-Cy5: a solution of NHS-Cyanine5 (0,0177 mmol, 1eq) in DMF was added to a solution of RGD-azide-QK (0,0177 mmol) in DMF (5mL) and the resulting mixture was stirred at r.t. overnight. After this time, diethyl ether (30mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 20 ml) with centrifugation (12500G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water at 4ºC and dialyzed against MQ water (3 x 25 L). Then the pH of the solution was adjusted to 7, the product was filtered (0.22 µm) and lyophilized to yield a blue recombinamer with 1eq of the lysine groups modified with Cy5 (RGD-azide-QK-Cy5).

RGD-azide-IKVAV: a solution of H-Cys[(3-succinimido-propionyl)-β-Ala-DBCO]-Gln-Ala-Ala-Ser-Ile-Lys-Val-Ala-Val-OH trifluoroacetate salt (0.02451 mmol, 2,2 eq) in DMF (0,5mL) was added to a solution of the RGD-azida in DMF (0.01114 mmol in 20 ml). The resulting mixture was stirred 8h. After this time, diethyl ether was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 ml) with centrifugation (12500G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water and dialyzed against

ultrapure water at 4ºC (3 x 25 L). Then the pH solution was measured and adjusted to 7, filtered and lyophilized to yield a white recombinamer with 1 eq of the azide groups modified with IKVAV (RGD-azide-IKVAV).

DRIR-cyclooctyne: (1R,8S,9S)-bicyclo [6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (126.6 mg, 9 eq.) was added to a solution of the ELR DRIR in DMF (2000 mg in 100 mL). The resulting mixture was stirred at room temperature (r.t.) for 60h. After this time, diethyl ether (300mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 mL) by centrifugation (12500 G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100 mL), dialyzed against ultrapure water at 4ºC (3 x 25 L), the pH of the solution was adjusted to 7, filtered (0.22 μm) and freeze-dryed to yield a white recombinamer with the 50% of the lysine groups modified with cyclooctyne (DRIR-cyclooctyne).

GTAR-cyclooctyne: (1R,8S,9S)-bicyclo [6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (126.6 mg, 9 eq.) was added to a solution of the ELR GTAR in DMF (2000 mg in 100 mL). The resulting mixture was stirred at room temperature (r.t.) for 60h. After this time, diethyl ether (300mL) was added to the mixture to give a white precipitate. The supernatant was removed and the solid was washed with acetone (3 x 100 mL) by centrifugation (12500 G, 10 min), dried under reduced pressure, redissolved in cold ultrapure water (100 mL), dialyzed against ultrapure water at 4ºC (3 x 25 L), the pH of the solution was adjusted to 7, filtered (0.22 μm) and freeze-dryed to yield a white recombinamer with the 50% of the lysine groups modified with cyclooctyne (GTAR-cyclooctyne).

4.2.3. Physicochemical characterization

4.2.3.1. High performance liquid chromatography (HPLC) amino acid analysis

The amino acid composition of the ELRs was analyzed by high-performance liquid chromatography (HPLC). Briefly, freeze-dried ELRs were hydrolyzed, derivatized with the AccQ-Tag Waters kit (Waters) and analyzed by HPLC coupled to a UV detector (HPLC 1200 Series, Agilent Technologies).

4.2.3.2. Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE)

The purity and the molecular weight of the employed ELRs was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, ELR solutions in ultrapure water (1 mg mL^{-1}) were denatured and homogeneously charged by the addition of SDS. This solutions were loaded, accompanied by an unstained marker (Fermentas), into a SDS-PAGE gel and run in a vertical electrophoresis system "MiniVE" of Hoefer (Amersham Pharmacia Biotec). Polyacrylamide gels were negatively stained with a 0.3 M copper chloride solution, imaged by a Gel Logic 100 Imaging System (Eastman Kodak) and analyzed with the Kodak 1D Image Analysis (Eastman Kodak) program.

4.2.3.3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF)

The molecular weight of the ELRs was analyzed by matrix-assisted laser desorption/ionization-Time-of-flight" (MALDI-TOF). Briefly, ELR solutions in ultrapure water or DMSO (1 mg mL^{-1}) were mixed with a 2,5-

dihydroxyacetophenone (DHAP) matrix and analyzed by a Bruker Daltonics Autoflex Speed (Bruker).

4.2.3.4. Proton nuclear magnetic resonance spectroscopy (1H-NMR)

The chemical groups of the ELRs were analyzed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). Briefly, ELR solutions in DMSO-D6 (20 mg mL^{-1}) were analyzed at 25°C in a Varian AV-400 instrument equipped with a One NMR probe (Agilent Technologies).

4.2.3.5. Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR)

The chemical bonding of the ELRs was analyzed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Briefly, freeze-dried ELRs were analyzed by a Fourier transform infrared Bruker Tensor 27 spectrophotometer equipped with a Golden gate ATR accessory (Bruker).

4.2.3.6. Differential scanning calorimetry (DSC)

Transition temperature (T_t) and enthalpy values associated to the endothermic phase transition of the ELRs in aqueous solution were analyzed by differential scanning calorimetry (DSC). Briefly, ELR solutions in ultrapure water (50 mg mL^{-1}) were analyzed by a DSC822e (Mettler Toledo).

4.2.3.7. Scanning electron microscopy (SEM)

Morphology of the ELR structures was analyzed by scanning electron microscopy (SEM). Briefly, liquid-nitrogen frozen and freeze-dried ELR structures were gold sputtered or directly imaged by a FEI Inspect F50 or a JEOL JSM-6500 (Fisher Scientific).

4.2.3.8. Environmental scanning electron microscopy (ESEM)

Morphology of the ELR structures was analyzed by environmental scanning electron microscopy (ESEM). Briefly, hydrated ELR structures were imaged by a Quanta FEG 250 (Fisher Scientific) or ESEM XL 30 FEG (Institute of Pathology, RWTH Aachen University Hospital, Germany).

4.2.3.9 Hydration analysis of ELR hydrogels

Hydration of ELR hydrogels was analyzed according to the following equation:

$$Gel\ hydration\ (\%) = \left(\frac{W_1 - W_2}{\rho_{water}}\right) * \frac{100}{V_{hydrogel}}$$

Briefly, hydrated (W_1) and lyophilized hydrogels (W_2) were weighted and the volume of the hydrated hydrogel ($V_{hydrogel}$) measured (ρwater is the density of water).

4.2.3.10 Rheological measurements

The elastic moduli of the ELR hydrogels were measure with 12 mm diameter hydrogels (260 µL), using time-sweep experiment at 37 °C, 1 % strain, 1 Hz frequency, 1000 µm gap (AR-2000ex rheometer, TA instruments). The selected conditions correspond to the linear viscoelastic region.

4.2.3.11 Equibiaxial analysis

Equibiaxial mechanical properties of the ELR venous valves was performed holding the sample in the radial and circumferential direction using a 30 mN preload before stretching the sample equibiaxially up to 7.5 N at a strain rate of 1% on a biaxial tester (Zwick Roell Biaxial Tester). Thickness of constructs was measured by microscope images (VHX-5000 digital microscope).

4.2.3.12 Burst strength studies

The structural failure pressure of ELR valves was analyzed in a burst chamber passing PBS at 7.5 mL/min over 1 cm2 samples until structural failure, using the pressure sensor (Jumo Midas pressure transmitter; JUMO GmbH & Co. KG).

4.2.3.13 Hydrodynamic testing

ELR venous valves were tested in a bioreactor system consisting of a reservoir, a pulsatile pump (702-6882, RS components), a video chamber and a unidirectional silicone rubber flow loop (Ismatec). Flow rates of 36.7% glycerol aqueous solution from 1 to 2 L/min and pressures from 40 to 75 mmHg were tested. Pressure sensors (Xtrans, Codan pvb Medical GmbH) were located before and after the ELR valve followed by a flow sensor (SonoTTTM Flowcomputer, em-tec GmbH).

4.2.3.14 Washout testing

Stagnation in ELR venous valves was analyzed in the hydrodynamic bioreactor using pulsatile (1500 mL/min) and steady (400 mL/min) flow conditions under peak column pressure of 60 mmHg. Dye pen solution in water 1:10 (v/v) was manually injected on each of the two bellies of the leaflets recording a video.

4.2.3.15 Balloon expandable dilatation catheter

Transcatheter delivery of crimped ELR venous valves was performed using the expandable balloon (PTA dilation catheter, Atlas Gold) and a silicon tube (Sigma Aldrich). The balloon was expanded up to 14 mm of diameter applying 6 atm of pressure with distilled water.

4.2.3.16. Histological analysis

Upon subcutaneous implantation in mice ELR hydrogels and 3D models were extracted and covered with optimum cutting temperature mounting medium (VWR). Then, samples were frozen in 2-methylbutane (Sigma Aldrich) cooled with liquid nitrogen and stored at -80 °C. Subsequently, samples were cut into 6-µm thick sections at -20 °C in a cryostat (Shandon Cryotome E Cryostat, Thermo scientific) and stained with hematoxylin and eosin solutions (Sigma Aldrich), obtaining images with a bright-field optical microscope (Nikon Eclipse 80i).

4.2.3.17 Immunohistochemistry

Cryostat micro-sections were fixed in acetone at -20 °C for 2 min, rinsed in PBS (3x), permeabilized with 0.1% TritonTM X-100 (Sigma-Aldrich), incubated 1 h at r.t. with 10% normal goat serum (Life Technologies), rinsed in PBS (3x), incubated O/N at 4 °C with the primary antibody diluted in 1% normal goat serum (in PBS), rinsed in PBS (3x), incubated 2 h at r.t. with secondary antibody diluted in 1% normal goat serum (in PBS) and mounted with fluoroshield mounting medium with DAPI (ab104139, Abcam). Images were with Nikon Eclipse Ti-E epifluorescence microscope (Nikon) and a Leica TCS SP8 LIGHTNING confocal microscope (Leica Microsystems).

4.2.3.18 Statistical analysis

The data was analyzed by a one-way analysis of variance (ANOVA) with post-hoc Holm–Sidak method considering significant a p-value of less than 0.05 (GraphPad Prism 7).

5. RESULTS

5.1. Publication 1: Combining tunable proteolytic sequences and a VEGF-mimetic peptide for the spatiotemporal control of angiogenesis within elastin-like recombinamer scaffolds

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Fernando González-Pérez, Arturo Ibáñez-Fonseca, Matilde Alonso, José Carlos Rodríguez-Cabello. Combining tunable proteolytic sequences and a VEGF-mimetic peptide for the spatiotemporal control of angiogenesis within Elastin-Like Recombinamer scaffolds. Acta Biomaterialia 2021, 130, 149–160. DOI: 10.1016/j.actbio.2021.06.005.

5.1.1. Abstract

One of the main challenges in regenerative medicine is the spatiotemporal control of angiogenesis, which is key for the successful repair of many tissues, and determines the proper integration of the implant through the generation of a functional vascular network. To this end, we have designed a three-dimensional (3D) model consisting of a coaxial binary elastin-like recombinamer (ELR) tubular construct. It displays fast and slow proteolytic hydrogels on its inner and outer part, respectively, both sensitive to the urokinase plasminogen activator protease. The ELRs used to build the scaffold included crosslinkable domains to stabilize the structure and a conjugated VEGF-derived peptide (QK) to induce angiogenesis. The mechanical and morphological evaluation of the ELR hydrogels proved their suitability for soft tissue regeneration. In addition, *in vitro* studies evidenced the effect of the QK peptide on endothelial cell spreading and anastomosis. Moreover, immunohistochemical analyses after subcutaneous implantation of the ELR hydrogels in mice showed the induction of a low macrophage response that resolved over time. The implantation of the 3D model constructs evidenced the ability of the fast proteolytic sequence and the QK peptide to guide cell infiltration and capillary formation in the pre-designed arrangement of the constructs. These results set the basis for the application of this type of scaffolds in regenerative medicine, where spatiotemporally controlled vascularization will help in the promotion of an optimal tissue repair.

5.2. Publication 2: Protease-sensitive, VEGF-mimetic peptide and IKVAV laminin-derived peptide sequences within elastin-like recombinamer scaffolds provide spatiotemporally synchronized guidance of angiogenesis and neurogenesis

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Fernando González-Pérez, Matilde Alonso, Israel González de Torre, Mercedes Santos, José Carlos Rodríguez-Cabello. Protease-Sensitive, VEGF-Mimetic Peptide and IKVAV Laminin-Derived Peptide Sequences within Elastin-Like Recombinamer Scaffolds Provide Spatiotemporally Synchronized Guidance of Angiogenesis and Neurogenesis. Advanced Healthcare Materials 2022, DOI: 10.1002/adhm.202201646.

5.2.1. Abstract

Spatiotemporal control of vascularization and innervation is a desired hallmark in advanced tissue regeneration. For this purpose, we design a 3D model scaffold, based on elastin-like recombinamer (ELR) hydrogels. This contains two interior and well-defined areas, small cylinders, with differentiated bioactivities with respect to the bulk. Both are constructed on a protease sensitive ELR with a fast-proteolyzed domain, but one bears a VEGF-mimetic peptide (QK) and the other a laminin-derived pentapeptide (IKVAV), to promote angiogenesis and neurogenesis, respectively. The outer bulk is based on a slow proteolytic sequence and RGD cell adhesion domains.

In vitro studies show the effect of QK and IKVAV peptides on the promotion of endothelial cell and axon spreading, respectively. The subcutaneous implantation of the final 3D scaffold demonstrates the ability to spatiotemporally control angiogenesis and neurogenesis *in vivo*. Specifically, the inner small cylinder containing the QK peptide promotes fast endothelialization, whereas the one with IKVAV peptide promotes fast neurogenesis. Both, vascularization and innervation take place in advance of the bulk scaffold infiltration. This scaffold shows that it is possible to induce vascularization and innervation in predetermined areas of the scaffold well ahead to the bulk infiltration. That significantly increases the efficiency of the regenerative activity.

5.3. Publication 3: Biohybrid elastin-like venous valve with potential for *in situ* tissue engineering

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Fernando González-Pérez, Sergio Acosta, Stephan Rütten, Caroline Emonts, Alexander Kopp, Heinz-Werner Henke, Philipp Bruners, Thomas Gries, José Carlos Rodríguez-Cabello, Stefan Jockenhoevel, Alicia Fernández-Colino. Biohybrid elastin-like venous valve with potential for in situ tissue engineering. Frontiers in Bioengineering and Biotechnology 2022, 10, 988533. DOI: 10.3389/fbioe.2022.988533.

5.3.1. Abstract

Chronic venous insufficiency (CVI) is a leading vascular disease whose clinical manifestations include varicose veins, edemas, venous ulcers, and venous hypertension, among others. Therapies targeting this medical issue are scarce, and so far, no single venous valve prosthesis is clinically available. Herein, we have designed a bi-leaflet transcatheter venous valve that consists of (i) elastin-like recombinamers, (ii) a textile mesh reinforcement, and (iii) a bioabsorbable magnesium stent structure. Mechanical characterization of the resulting biohybrid elastin-like venous valves (EVV) showed an anisotropic behavior equivalent to the native bovine saphenous vein valves and mechanical strength suitable for vascular implantation. The EVV also featured minimal hemolysis and platelet adhesion, besides actively supporting endothelialization *in vitro*, thus setting the basis for its application as an *in situ* tissue engineering implant. In addition, the hydrodynamic testing in a pulsatile bioreactor demonstrated excellent hemodynamic valve performance, with minimal regurgitation (< 10%) and pressure drop (< 5 mmHg). No stagnation points were detected and an *in vitro* simulated transcatheter delivery showed the ability of the venous valve to withstand the implantation procedure. These results present a promising concept of a biohybrid transcatheter venous valve as an off-the-shelf implant, with great potential to provide clinical solutions for CVI treatment.

6. CONCLUSIONS AND FUTURE PROSPECTS

6.1. Combining tunable proteolytic sequences and a VEGF-mimetic peptide for the spatiotemporal control of angiogenesis within elastin-like recombinamer scaffolds

The combination of fast and slow proteolytic sequences and the QK pro-angiogenic peptide into a 3D ELR system confirmed the spatiotemporal control of angiogenesis. Crosslinkable domains were included to stabilize the structure. Mechanical and morphological analysis identified their applicability as soft tissue substitute. *In vitro* evaluation proved the QK effect over endothelial cell spreading. Subcutaneous implantation in mice demonstrated the ability of the 3D model to spatiotemporally guide cell infiltration and vascularization in the pre-programed orientation. The combination of protease-sensitive and pro-angiogenic domains enabled a synergistic effect over the promotion of angiogenesis. A mild immunogenic response with shift towards pro-healing macrophage was also confirmed. Thus, the developed ELRs demonstrated their applicability for the fabrication of 3D models where the angiogenesis process can be spatiotemporally tuned on request.

6.2. Protease-sensitive, VEGF-mimetic peptide and IKVAV laminin-derived peptide sequences within elastin-like lecombinamer scaffolds provide spatiotemporally synchronized guidance of angiogenesis and neurogenesis

Spatiotemporal control of innervation and angiogenesis was achieved in a 3D model ELR system that combined fast and slow proteolytic sequences, the QK pro-angiogenic peptide and the IKVAV pro-innervation peptide. The fabricated 3D model consisted of a pair of internal cylinders on which one bears the QK peptide and the other the IKVAV peptide, displaying both fast protease-sensitive sequences. Catalyst-free click-crosslinkable domains were

incorporated to provide morphological and mechanical stability similar to that used for soft tissue regeneration. *In vitro* studies confirmed the IKVAV effect over C6 glial cells neurite outgrowth. *In vivo* analysis of the 3D models implanted subcutaneously in mice demonstrated the independent and simultaneous spatiotemporal promotion of angiogenesis or neurogenesis on each of the internal cylinders. Furthermore, a synergistic effect was confirmed between the proteolytic and QK and IKVAV bioactive signals. Therefore, the fabricated ELRs confirmed their use for the development of scaffolds with pre-programed vascularization and innervation in space and time.

6.3. Biohybrid elastin-like venous valve with potential for *in situ* tissue engineering

A ready-to-use cell-free transcatheter venous valve substitute for treating chronic venous insufficiency (CVI) was developed from a protease-sensitive ELR hydrogel, a textile mesh and a bioabsorbable magnesium stent. Mechanical characterization confirmed the anisotropic behavior and peak tangent moduli similar to that of the native bovine vein valves, and a strength suitable for implantation. *In vitro* studies demonstrated non-thrombogenic, hemocompatible and self-endothelialization properties. Hydrodynamic testing proved excellent hemodynamic performance, whereas washout studies confirmed no stagnation points and *in vitro* simulated transcatheter delivery identified the maintenance of functionality. Overall, the developed ELR transcatheter venous valve provides a promising concept of a bioresorbable substitute for future animal *in vivo* studies and clinical translation to fill the existing gap in comercially available prosthesis for CVI treatment.

6.4. Future prospects

The thesis presented herein delve into unexplored areas of vascular, nervous and venous valve research, enlarging the well-known extremely powerful versatility of the bioinspired ELRs. In the first publication, a 3D scaffold displaying protease-sensitive, cell-adhesive and a VEGF mimetic peptide in a pre-designed orientation was synthesized, demonstrating the spatiotemporal control of cell infiltration and vascularization *in vivo*. For the second publication, a 3D model containing protease-sensitive, cell-adhesive, a VEGF mimetic peptide and a IKVAV pro-innervation peptide in a two cylinder design was fabricated, demonstrating the spatial and temporal guidance of the angiogenesis and neurogenesis *in vivo*. In the third publication, a transcatheter bioabsorbable venous valve exhibiting protease-sensitive and cell-adhesive peptides was developed, confirming its suitability for a ready-to-use off-the-shelf venous valve replacement substitute. The results obtained herein not only represent a small brick in the development of science, but also set the basis and encourage the subsequent investigations based on these smart ELRs building blocks for TERM. For example, the fabrication of scaffolds that can pre-define the vascularization and innervation process in space and time envision their potential application for organoid development where complex structures coexist in the same system. Thus, taking advantage of the modular design of the ELRs, novel designs including the zipper, Bombyx mori silk fibroin or self-assemble peptide sequences can be developed conferring the necessary bioprinting properties for their use as advanced bioinks. In addition, depending on the specific application, other biofunctionalities such as antimicrobial resistance or drug-depots could be incorporated. This will allow to reproduce in high detail native tissues and organs for their final clinical translation to fill the existing gap in organ repair and replacement. On the other hand, the developed venous valve paves the way for the design and fabrication of adult

and child venous and heart valves. In particular, systems including a silk fibroin textile mesh instead of the PET textile mesh would benefit from a better biocompatibility and absorbability, improving the cell-mediated remodeling and integration. Additionally, ELR scaffolds displaying higher degrees of crosslinking, concentration or self-assembling peptides will be necessary in the case of heart valves, where improved mechanical properties are required. Alternatively, venous and heart valves with tailored degradation kinetics, as well as antimicrobial resistance could be envisaged depending on the patient-specific variabilities, all in all rendering a scaffold that will faithfully replicates the native tissue, and helps in the current necessity for proper substitutes in clinics. Last but not least, the potential of these biomimicking materials rely on the merge of different areas of knowledge, namely chemistry, physics, biology, engineering, computational and information technologies, and the novel techniques that are in continue development. Thus, progressively fulfilling the gaps related to the inner nature of the human body and the intriguing and continue developing natural environment.

7. APPENDIX

7.1. Abbreviations

AA: Amino acid

ANOVA: Analysis of variance

BSA: Bovine serum albumin

CAD: computer aid design

DLS: Dynamic light scattering

DMEM: Dulbecco's Modified Eagle Medium

DMSO: dimethyl sulfoxide

DNA: Deoxyribonucleic acid

DSC: Differential scanning calorimetry

ECM: Extracellular matrix

ECs: Endothelial cells

EDTA: Ethylenediaminetetraacetic acid

ELP: Elastin-like polypeptide

ELR: Elastin-like recombinamers

ESEM: Environmental scanning electron macroscopy

FBS: Fetal bovine serum

FTIR: Fourier Transform Infrared spectroscopy

HPLC: High-performance liquid chromatography

HUVECs: Human umbilical vein endothelial cells

H&E: hematoxylin-eosin

IDP: Intrinsically disordered protein

ITC: Inverse transition cycle

ITT: Inverse temperature transition

LB: Luria-Broth

LCST: Lower critical solution temperature

LOX: Lysyl oxidase

MALDI-TOF: Matrix-assisted laser desorption/ionization-time-of-flight

MMP: Matrix metalloproteinase

MQ: MilliQ ultra-pure water

MW: Molecular weight

NMR: Nuclear magnetic resonance

PA: Plasminogen activator

PBS: Phosphate buffered saline

pD: pDrive

PEG: poly(ethylene glycol)

PGA: polyglycolic acid

pKa: Dissociation constant of acids

pKb: Dissociation constant of bases

PLA: poly(L-lactic acid)

PLGA: poly(co-glycolic lactic acid

SD: Standard deviation

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SELR: Silk-elastin-like recombinamer

SEM: Scanning electron microscopy

SPAAC: Strain-promoted alkyne-azide cycloaddition

TB: Terrific Broth medium

THPC: Tetrakis (hydroxymethyl) phosphonium chloride

TE: tissue engineering

TEMED: Tetramethylethylenediamine

TERM: tissue engineering and regenerative medicine

Tt: Transition temperature

tPA: tissue plasminogen activator

uPA: Urokinase plasminogen activator

VEGF: vascular endothelial growth factor

7.2. Amino acid nomenclature

Amino acid	3-letter code	1-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G

Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

7.3. Author's contributions

7.3.1. Publications

2022

- **Fernando González-Pérez**, Matilde Alonso, Israel González de Torre, Mercedes Santos, José Carlos Rodríguez-Cabello. **Protease-Sensitive, VEGF-Mimetic Peptide and IKVAV Laminin-Derived Peptide Sequences within Elastin-Like Recombinamer Scaffolds Provide Spatiotemporally Synchronized Guidance of Angiogenesis and**

Neurogenesis. Advanced Healthcare Materials 2022, DOI: 10.1002/adhm.202201646.

- **Fernando González-Pérez**, Sergio Acosta, Stephan Rütten, Caroline Emonts, Alexander Kopp, Heinz-Werner Henke, Philipp Bruners, Thomas Gries, José Carlos Rodríguez-Cabello, Stefan Jockenhoevel, Alicia Fernández-Colino. **Biohybrid elastin-like venous valve with potential for *in situ* tissue engineering**. Frontiers in Bioengineering and Biotechnology 2022, 10, 988533. DOI: 10.3389/fbioe.2022.988533.
- Arturo Ibáñez-Fonseca, Ana Rico, Silvia Preciado, **Fernando González-Pérez**, Sandra Muntión, Jesús García-Briñón, María-Carmen García-Macías, José Carlos Rodríguez-Cabello, Miguel Pericacho, Matilde Alonso and Fermín Sánchez-Guijo. **Mesenchymal Stromal Cells Combined With Elastin-Like Recombinamers Increase Angiogenesis *In Vivo* After Hindlimb Ischemia**. Frontiers in Bioengineering and Biotechnology 2022, 10:918602. DOI: 10.3389/fbioe.2022.918602.
- Israel González de Torre, **Fernando González-Pérez**, Julio Fernández-Fernández, Irene Montequi, José Carlos Rodríguez-Cabello. **Smart protein-polymers as tunable and bioactive bioinks**. Advanced Drug Delivery Reviews 2022 under review.
- Ricardo Usategui-Martín, Kevin Puertas-Neyra, Nadia Galindo-Cabello, Leticia A Hernández-Rodríguez, **Fernando González-Pérez**, José Carlos Rodríguez-Cabello, Rogelio González-Sarmiento, José Carlos Pastor, Iván Fernandez-Bueno. **Retinal Neuroprotective Effect of Mesenchymal Stem Cells Secretome Through Modulation of Oxidative Stress, Autophagy, and Programmed Cell Death**.

Investigative Ophthalmology & Visual Science 2022; 63(4):27. DOI: 10.1167/iovs.63.4.27.

- Kevin Puertas-Neyra, Nadia Galindo-Cabello, Leticia A. Hernández-Rodríguez, **Fernando González-Pérez**, José Carlos Rodríguez-Cabello, Rogelio González-Sarmiento, José Carlos Pastor, Ricardo Usategui-Martín and Ivan Fernandez-Bueno. **Programmed Cell Death and Autophagy in an *in vitro* Model of Spontaneous Neuroretinal Degeneration**. Frontiers in Neuroanatomy 2022, 16:812487. DOI:10.3389/fnana.2022.812487

2021

- **Fernando González-Pérez**, Arturo Ibáñez-Fonseca, Matilde Alonso, José Carlos Rodríguez-Cabello. **Combining tunable proteolytic sequences and a VEGF-mimetic peptide for the spatiotemporal control of angiogenesis within Elastin-Like Recombinamer scaffolds**. Acta Biomaterialia 2021, 130, 149–160. DOI: 10.1016/j.actbio.2021.06.005
- José Carlos Rodríguez-Cabello, Israel González de Torre, Miguel González-Pérez, **Fernando González-Pérez**, Irene Montequi. **Fibrous Scaffolds From Elastin-Based Materials**. Frontiers in Bioengineering and Biotechnology 2021, 9:6523849. DOI: 10.3389/fbioe.2021.652384.
- Lubinda Mbundi, Miguel González-Pérez, **Fernando González-Pérez**, Diana Juanes-Gusano, José Carlos Rodríguez-Cabello. **Trends in the Development of Tailored Elastin-Like Recombinamer-based Porous Biomaterials for Soft and Hard Tissue Applications**. Frontiers in Materials 2021, 7:601795. DOI: 10.3389/fmats.2020.601795.

7.3.2. Conferences

2022

- Participation in the international congress “**ESB (European Society for Biomaterials) 2022**”, 4-8 September 2022, Bordeaux, France with the oral presentation of the work: "Combination of proteolytic sequences, VEGF-mimetic peptide and laminin-derived peptide for the spatiotemporal direction of angiogenesis and neurogenesis in Elastin-Like Recombinamer scaffolds"
- Participation in the international congress “**ESB (European Society for Biomaterials) 2022**”, 4-8 September 2022, Bordeaux, France as author in the poster: "Biohybrid miniaturized valves based on Elastin-like recombinamers"
- Participation in the international congress “**Termis-EU 2022 (Tissue Engineering and Regenerative Medicine International Society)**”, 28 June-1 July 2022, Krakow, Poland with the oral presentation of the work: "Combining proteolytic sequences, VEGF-mimetic peptide and laminin-derived peptide within Elastin-Like Recombinamer scaffolds for the spatiotemporal direction of angiogenesis and neurogenesis"
- Participation in the congress “**II Encuentro de Jóvenes Investigadores de la Universidad de Valladolid**”, 23 June 2022, Valladolid, Spain with the oral presentation of the poster: "Combination of QK peptide and protease-sensitive sequences in Elastin-Like Recombinamer (ELR) hydrogels for the spatiotemporal guidance of angiogenesis"
- Participation in the international congress “**GEP-SLAP 2022 (XVI Reunión del Grupo Especializado de Polímeros – GEP 2022 y XVII Simposio Latinoamericano de Polímeros - SLAP 2022)**”, 8-12 May

2022, San Sebastián, Spain with the presentation of the poster: "Role of QK peptide and proteolytic sequences in the promotion and direction of angiogenesis in Elastin-Like Recombinamer (ELR) hydrogels", obtaining the "Best Poster Award" in the conference

2021

- Participation in the international congress “**ESAO/TERMIS ONLINE Winter School**”, 24-26 February 2021, Jaca, Spain with the oral presentation of the work: "Use of proteolytic sequences and QK peptide in elastin-like recombinamer (ELR) hydrogels for the direction and promotion of angiogenesis"
- Online attendance at the international congress “**EPF (European Polymer Congress) Inter-Congress Workshop**”, 28 June 2021, Prague, Czech Republic
- Participation in the congress “**31st Conference of the European Society for Biomaterials (ESB) held together with the 43rd Annual Congress of the Iberian Society of Biomechanics and Biomaterials (SIBB)**” 5-9 September 2021, Porto, Portugal, with the oral communication of the work: “Combination of QK peptide and proteolytic sequences for the promotion and direction of angiogenesis in Elastin-Like Recombinamer (ELR) hydrogels”
- Online attendance at the international workshop “**EMBO (European Molecular Biology Organization) Designing functional biomolecular assemblies: Beyond biology**”, 28 September to 1 October 2021, Bled, Slovenia
- Participation in the congress “**ESVO The European Society of Veterinary Ophtalmology Conference**”, 2-3 October 2021, Berlin,

Germany, with the póster: "Retinal cells apoptosis characterization during *in vitro* porcine retinal neurodegeneration"

- Online attendance at the international congress "**XIV annual Cyber conference**" 15-16 November 2021, Madrid, Spain

2019

- Participation in the congress "**TERMIS European Chapter Meeting, Tissue Engineering Therapies: From Concept to Clinical Translation & Commercialisation**" 27-31 May 2019, Rhodes, Greece, with the oral communication of the work: "Photo-crosslinkable mono-component Elastin -Like Recombinamer bioink for 3D printing"
- Participation in the international congress "**ESB 2019 30th Annual conference of the european Society for biomaterials**", 9-13 September 2019, Dresden, Germany. With the work: "Elastin-Like Recombinamers as a new generation of bioinks for 3d bioprinting"
- Participation in the international congress "**2Iwamha 2019 2nd International workshop on advanced materials for healthcare applications**", 8-9 October 2019, Funchal, Madeira, Portugal. With the poster: "Use of proteolytic sequences and QK peptide as a way to promote and direct angiogenesis in Elastin-Like Recombinamer (ELR) hydrogel"
- Participation in the congress "**SIBB 2019 XLII Congress of the Iberian Society of Biomechanics and Biomaterials**", 15-16 November 2019, Madrid, Spain. With the oral communication of the work: "QK peptide and proteolytic sequences in elastin-like recombinamer (ELR) hydrogels as a way to direct and enhance angiogenesis".

- Participation in the workshop “**Biomapp 2019 3rd Biennial Young Researchers Workshop on Biomaterials and Applications**”, 4-5 December 2019, Leioa, Bilbao, Spain. With the poster: “Combination of proteolytic sequences and QK peptide in elastin-like recombinamer (ELR) hydrogels for the promotion and direction of angiogenesis”

2018

- Participation in the “**I Research Conference on Bioengineering and Medicine**”, University of Valladolid March 22, 2018
- Collaboration in the "**Open Doors Day of the University of Valladolid**", April 13, 2018
- Participation with oral communication in the “**Second Bioforge Group Workshop: Organoids**”, University of Valladolid June 6, 2018
- Participation as organizer and speaker in the workshop "**From bacteria to your body**", during the "Thirteenth European Night of Researchers" held at the Science Museum of Valladolid on September 28, 2018

7.4. Internships

2021

- 5 months internship at the laboratory of **Biohybrid & Medical Textiles (BioTex)** of the **University RWTH of Aachen, Germany**, under the supervision of Stefan Jockenhoevel, Professor Dr. Med. and Head of the Department of Biohybrid & Medical Textiles (BioTex), from the 15 of July to the 19 of December of 2021.

7.5. Courses and certifications

- **“Front cover”** of Advanced Healthcare Materials with the article “Protease-Sensitive, VEGF-Mimetic Peptide and IKVAV Laminin-

Derived Peptide Sequences within Elastin-Like Recombinamer Scaffolds Provide Spatiotemporally Synchronized Guidance of Angiogenesis and Neurogenesis" DOI: 10.1002/adhm.202201646

- "**Best Poster Award**" at the international congress "GEP-SLAP 2022", 8-12 May 2022, San Sebastián, Spain with the presentation of the poster: "Role of QK peptide and proteolytic sequences in the promotion and direction of angiogenesis in Elastin-Like Recombinamer (ELR) hydrogels"
- Certification "*In vivo* experimentation for the design and realization of animal trials"
- Online course Care of Animals Function A 34 hours, Euthanasia of Animals Function B 24 hours, and Performance of Procedures Function C 50 hours for all the species of Annex II taught by Animalaria Formación y Gestión, S.L. held from January 10 to March 7, 2022. Recognized by the General Directorate of Agriculture and Livestock of the Community of Madrid
- Online course Introduction to Fiji/Image J of 14 hours for fluorescence image analysis, offered through the Virtual Campus of Hospital Sant Joan de Déu Barcelona from October 21 to November 6, 2020.
- Online course on Spectral Confocal Microscopy and Super-Resolution, Theoretical-Practical of 12 hours given at the Hospital Sant Joan de Déu Barcelona from June 7 to 10, 2021
- Training course of the Leica SP8 3X Confocal Microscope with FALCON (FLIM) held at the University of Valladolid on September 23 and 24, 2019. Operation of the TCS-SP8 Confocal system, LIGHTNING Super resolution system and the FALCON (FLIM) system.

7.6. Teaching

2021, 2020 and 2019

- Teaching activities in the Master in Physics at the University of Valladolid.
- Teaching activities in the Degree in Mechanical Engineering and Biomedical Engineering at the University of Valladolid (Spain).

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