RECOMBINANT HYBRID PROTEIN AND ITS USE

Field of the Invention

The invention relates to a recombinant hybrid protein REC2z with a structure as defined in the description, recombinant DNA sequences encoding the protein, vectors containing the recombinant DNA sequences, and eukaryotic and prokaryotic expression systems using the in vitro recombinant DNA sequences described below. The invention also relates to the use of the recombinant hybrid protein REC2z as a component of bioink for bioprinting and also as a component of biomaterials. In addition, the invention relates to the use of recombinant DNA sequences, expression vectors and expression systems to produce the recombinant hybrid protein REC2z. The invention can be implemented in bioprinting, medicine and cell and tissue engineering.

Prior Art

WO2024014972A2 describes recombinant hybrid proteins, their DNA sequences, vectors for their expression and their uses in biotechnology and regenerative medicine. Two major recombinant hybrid proteins, RE15mR and EJ17zipR, consisting of different combinations of functional domains such as resilin, elastin, silk fibroin, RGD motifs (recognised by integrins), MMP motifs (recognised by matrix metalloproteinases), lysine-rich (K+) domains allowing chemical modifications, and ZIP domains stabilising the supramolecular structure, are described in detail.

EP3868779A1 describes recombinant elastin-like ELR biopolymers, composed of monomers containing domains present in natural elastin, as well as monomers containing a sequence called 'silk' and/or monomers containing a sequence called HLF, belonging to a natural class of proteins called zippers, which are non-toxic and therefore suitable for use as bioinks. The subject-matter described relates in particular to compositions containing the aforementioned biopolymers used as bioink for 3D printing. Through production by recombinant DNA technology, the amino acid structure of ELRs can be designed to modulate the ability of biopolymers to cross-link or self-organise allowing the formation of physical and/or chemical hydrogels, with a subsequent improvement in mechanical properties. The production of ELR biopolymers disclosed in this subject-matter is carried out using recombinant DNA technology, which involves constructing multivalent genes and then they are used to produce biopolymers in microorganisms, such as *Escherichia coli* bacteria, or in other expression systems.

EP4294832A1 describes the general principles and technologies used to produce recombinant proteins, including the use of adeno-associated vectors (AAVs) and plasmid expression systems with the T7 phage promoter and the creation of fusion proteins with elastin-like polypeptides (ELPs) and resilin-like polypeptides (RLPs). The possibility of producing a wide range of proteins, depending on which gene sequence is inserted into the AAV vector, is described. In particular, a process for obtaining the protein containing the following steps is disclosed: (i) construction of an expression vector (in the case of proprietary fusion proteins, the gene encoding the ELP or RLP is

a gene combined with the genetic sequence of another protein to form a single sequence encoding the fusion protein); (ii) introduction of the vector into host cells [this can be achieved by transformation (*for E. coli*) or transduction (for mammalian cells by means of AAV)]; (iii) expression of the protein; (iv) purification of the protein.

Proteins that can be obtained by this process include: ELP or RLP fusion proteins, which may have enhanced stability, solubility or other desirable physicochemical properties to facilitate their use in medicine, research or biotechnology; therapeutic proteins such as enzymes, growth factors, antibodies, signalling proteins or other proteins with potential medical applications.

According to one embodiment, various proteins with biological functionality are encoded. These proteins were fused to sequences encoding phase transition behaving polypeptides and produced as recombinant fusion proteins in *E. coli* BL21 cells. Specifically, the sequences encoding the fusion protein were cloned into the pET24 plasmid, which was then transformed into *E. coli* cells. Fusion protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a shake flask containing cell culture. Fusion proteins were purified using phase separation, indicating a yield of at least 10 mg/L.

US8470967B2 discloses a method of biopolymer synthesis that is based on molecular biology techniques, using diverse motifs such as elastin, collagen and resilin to form elastomers with specific properties. Elastin contains VPGXG motifs that influence its flexibility. Collagen is characterised by the presence of PGX motifs, where X is any amino acid, which influences the formation of three-dimensional structures. Resilin, with motifs like AQTPSSQYGAP, shows exceptional flexibility and the ability to return to its original shape. Two types of vectors were used to synthesise the biopolymers described here: a modified pET25 vector and a modified pET24 vector. These vectors were used to transform *E. coli* cells, allowing the expression of synthesised biopolymers. The method described includes the following steps: (i) design of single-stranded DNA sequences encoding 1 to 5 copies of an amino acid motif; (ii) cyclic ligation of designed sequences and their amplification by means of specific primers; (iii) gene polymerisation by circular amplification and overlapping elongation; (iv) insertion of oligomers into modified pET25 or pET24 vectors and transformation of *E. coli* cells. After 6-7 hours of growth, expression was induced by addition of IPTG to a final concentration of 1 mM; (iv) clonal screening and DNA sequencing; (v) expression of proteins in *E. coli* and their purification; (vi) characterisation of the phase transition temperature of biopolymers.

The resulting biopolymers can be used to create 'smart' protein polymers with flexibility and sensitivity to environmental changes such as temperature or pH. Thanks to these properties, they can, for example, promote tissue growth and regeneration by utilising specific amino acid motifs in elastin, collagen and resilin.

EP3979873A4 describes methods for producing recombinant silk proteins. These methods aim to replicate and enhance the unique properties of natural silk proteins, such as those from silkworms and spiders, for a variety of applications, including biomaterials, textiles and medical

devices. Recombinant silk proteins are synthetic proteins produced heterologously in prokaryotic (e.g. *E. coli*) or eukaryotic expression systems by genetic engineering methods. These proteins can mimic the properties of natural silk proteins, but can be customized for specific applications. The process of producing these proteins involves the following steps: (i) design of the genetic sequence; (ii) insertion of this sequence into a recombination vector, wherein plasmids, bacteriophages and viruses can serve as such vectors; plasmid expression vectors are particularly valued for their ability to express the target gene in the host cell and their ability to self-replicate; when using *Escherichia coli* strain Rosetta (DE3) as a host, plasmid vectors such as pET22b(+) and pCold are recommended, with pET22b(+) being considered for its efficiency in protein production; (iii) transformation; (iv) expression and purification.

The description includes information on the production of recombinant silk proteins and the potential for integrating such proteins with others, including resilin, elastin and collagen.

EP3955886A4 describes methods for producing recombinant silk proteins using recombinant DNA technology. This involves the use of genetic engineering to produce silk proteins (both mulberry silk and spider silk), in the expression systems of microorganisms such as bacteria (e.g. *Escherichia coli*) or other systems such as yeast, plant or animal cells. The production process described includes the following steps: gene design and synthesis, cloning and transformation, protein expression, protein purification, formulation. The purified proteins can then be used in the formulation of final products such as fibres, hydrogels, films or other materials used in cosmetics, biomedicine or industrial materials.

Recombinant silk proteins are characterised by their high biocompatibility, biodegradability, strength and flexibility, making them attractive for various industrial and biomedical applications. Specific protein structural domains have been mentioned as components that can be combined with spider silk proteins to form hybrid spider silk proteins.

WO2023096903A1 describes methods for the production of recombinant elastomeric proteins such as resilin, elastin and collagen, which can be incorporated into cellulose fibre compositions to improve their viscoelastic properties. The main purpose of adding these proteins to cellulose is to create fibres with better mechanical properties that can find applications in various industries, including textiles and biomedicine. Recombinant proteins such as resilin, elastin and - potentially - collagen are produced using recombinant DNA technology. These proteins are expressed in microbial host systems, such as *E. coli* bacteria, and then isolated and purified using chromatographic techniques. The addition of recombinant elastomeric proteins to cellulose fibre compositions is a promising method for improving their mechanical properties, opening up new possibilities for material applications. Resilin, elastin and potentially collagen play key roles in achieving better flexibility, resilience and overall fibre strength, which could contribute to the development of more advanced and sustainable materials in the textile industry, biomedical industry and other fields.

US20230128695A1 details processes and methods for producing synthetic polymers containing elastin, resilin and collagen sequences to form polymeric systems used in a variety of fields, including regenerative medicine, textiles or construction materials. The design and modification of recombinant structural proteins uses genetic engineering to create new or modified structural proteins such as elastin, resilin or collagen to achieve desired properties such as greater flexibility, strength or the ability to self-regenerate. These techniques allow the precise design of amino acid sequences that determine the functionality of the final product. The publication details the use of these proteins to create new materials, emphasising that they can be used alone or in combinations to create a wide range of materials with diverse properties. In the context of the production process, the described proteins can be produced in expression systems such as bacteria (e.g. *E. coli*), yeast, insect cells or even in plant systems, depending on the optimisation of the process and the required post-translational modification of the protein. The process involves key steps such as cloning the gene encoding the artificial fibroin into a suitable expression vector, transformation into a host organism, cell cultures, expression of the protein, followed by purification and characterisation. Examples of vectors include pBTrp2, pBTac1, pET22b, pCold, and many others, depending on the purpose of the experiment and the host cell chosen. A bacterial culture procedure is also described, where a solution containing glucose and yeast extract was added after the glucose from the medium had been used up, maintaining certain conditions (temperature, pH, dissolved oxygen concentration) for 20 hours. The expression of artificial fibroin was then induced by adding IPTG and, after another 20 hours, bacterial cells were harvested for SDS-PAGE analysis, confirming the expression of the target protein.

EP4087616A1 concerns the process of creating recombinant proteins, with a particular focus on artificial fibroins. Recombinant proteins are produced by genetic engineering methods, whereby genes encoding the desired protein are inserted into a host organism (e.g. bacteria, yeast, plant or animal cells), which then produces the protein. Here, the different types of synthetic fibroin designed and manufactured using such technologies are described in detail. Resilin- and elastin-derived polypeptides play key roles as thermosensitive polypeptides that can be used in protein engineering and nanotechnology for medical and biotechnological purposes. The document describes the detailed process of creating artificial fibroin, a type of recombinant protein, by genetic engineering, which includes: gene design and synthesis, cloning into expression vectors, transformation and expression in host cells, and protein purification.

WO2023038154A1 describes the formation of a polypeptide fibre using a method involving combining multiple raw fibres containing artificial polypeptides using a binder and enzyme. A key element of this process is the use of enzymes, such as oxidases, which react with at least one of the components - a synthetic polypeptide or binder - leading to the formation of interconnected polypeptide fibres. Sequences derived from resilin and elastin, can be combined to create synthetic polypeptide fibres or recombinant proteins. By combining these two thermosensitive polypeptides, materials can be created that take advantage of the unique properties of both

polypeptides, i.e. the exceptional flexibility of resilin and the thermosensitivity of elastin. As a result, the described method enables the creation of polypeptide materials that can find applications in the fields of biomaterials, tissue engineering and technologically advanced textiles, among others.

WO2019030524A1 relates to the process of producing recombinant proteins, with a particular focus on fusion proteins containing adhesion motifs derived from fibronectin. These proteins have been designed to promote stem cell self-renewal, which is particularly important in biomedical contexts, e.g. in regenerative therapy and tissue engineering. Fusion proteins are created by genetic engineering methods, which involves combining genes encoding different protein domains into a single continuous DNA fragment, which is then expressed in expression systems (e.g. in bacterial cells) to produce the desired recombinant proteins. In this case, one part of the fusion protein is the adhesion protein domain (for example, a fibronectin fragment containing the RGD sequence) and the other part is the polymerisation domain (for example, the immunoglobulin Z1 and Z2 domains derived from titin), allowing the formation of polymeric structures capable of promoting stem cell adhesion and self-renewal. The document mentions the use of elastin-like polypeptides (ELPs), which are a type of elastin-inspired thermosensitive polymers. Elastin-like polypeptides can change their physicochemical properties in response to temperature changes, allowing them to be used as materials to create structures capable of controlled drug release or as components of frameworks for tissue engineering.

US20180071434A1 relates to a hydrogel obtained from recombinant proteins, among others. In particular, the use of both resilin and elastin peptide sequences to create scaffolds with specific mechanical properties such as elasticity, flexibility and the ability to form structures with specific shapes was disclosed. These properties could be important in many applications such as tissue engineering, biotechnology or regenerative medicine.

EP1948684A1 relates to genetically modified yeast producing a recombinant collagen-like protein, specifically a recombinant protein bound to the mussel byssus. The yeast was modified by introducing expression vectors encoding a recombinant protein and proline-4-hydroxylase (P4H), which is required to stabilise the collagen helix by catalysing the hydrolysis of proline to hydroxyproline. Consistent with this disclosure, the resilin and elastin sequences play a key role in providing flexibility to the recombinant protein, as in natural mucilage collagen. This is important for the potential applications of this protein in regenerative medicine, such as the production of surgical threads, prosthetic materials or dressings that require flexibility and elasticity. The described production of recombinant collagen-like protein includes the steps of expression vector preparation, yeast transformation, protein expression, protein recovery.

EP0803574A2 discloses a method for the production of recombinant proteins, comprising the following steps: (i) construction of expression vectors [creation of recombinant vectors that contain the retrovirus Gag protein gene fused to a heterologous gene encoding the protein of interest; the *gag* gene is responsible for producing the viral virion-forming protein that enables its

secretion from the cell]; (ii) modification for myristylation [introducing a modification in the *gag* gene so that the protein produced is myristylated; myristylation is the addition of myristic acid to a protein, which increases its interaction with cell membranes and facilitates secretion]; (iii) genetic modification of host cells [introducing a constructed vector into host cells (e.g. mammalian cells) by transduction, transfection or other genetic techniques]; (iv) recombinant protein expression [cultivation of host cells under appropriate conditions, which allows expression of Gag-heterologic protein fusions and their appropriate post-translational modification, including myristoylation]; (v) formation and secretion of virus-like particles, fusion proteins are secreted from the cell because these particles structurally resemble viral particles but are not infectious].

US8288347B2 describes the formation of hydrogels from silk fibroin, which can be enriched with domains of various proteins such as resilin, elastin and collagen to obtain desired material properties such as hardness, flexibility and resistance to biodegradation. Among other things, the production of hydrogels uses chemical and physical crosslinking techniques, as well as controlled mixing of components to achieve specific properties of bioinspired materials that can be used in various biomedical applications.

US9815874B2 relates to isolated monomers of fibrous proteins such as resilin, elastin, spider silk, mulberry silk, collagen and sea byssus protein that are linked to the hyaluronic acid binding domain via a polypeptide bond. This publication also describes the formation of isolated composites consisting of a fibrous protein monomer and hyaluronic acid that are not attached to a solid substrate. The composites described can include elastin, resilin and collagen. These fibrous proteins have been listed as selected monomers that can be used to create new composite materials in combination with polysaccharides such as hyaluronic acid.

US10925999B2 disclosed cross-linked hydrogels containing protein polymers, including silk fibroins with phenolic side chains. The process described involves combining an aqueous solution of silk fibroin with peroxidase and peroxide, where the hydrogel is characterised by a beta-card secondary structure and structure-crosslinking bonds formed by the phenolic side chains of the silk fibroin. In US10925999B2, resilin, elastin and collagen act as components of hydrogels which find applications in tissue engineering and tissue regeneration. Elastin and collagen are natural protein polymers that provide flexibility and structural strength to the hydrogel, which promotes support for cell growth and functioning. Resilin, with its unique elastic properties, can further enhance the elasticity and ability of the hydrogel to recover its shape after deformation. These biopolymers are key to adjusting the mechanical and degradation properties of hydrogels, which has applications in tissue engineering and regenerative medicine.

The publication by Bracalello A. et al. ('Design and production of a chimeric resilin-, elastin-, and collagen-like engineered polypeptide', 2011, Biomacromolecules, 12, 8, 2957-2965, doi: 10.1021/bm2005388.) describes the design, production and initial characterisation of a chimeric

polypeptide that combines sequences derived from highly flexible proteins, such as resilin and elastin, and collagen-like sequences.

In turn, the publication by Nuc P. and K. ('Produkcja rekombinowanych bialek w *Escherichia coli*' 2006, Postepy Biochemii 52 (4), 448-446), discloses preparation methods that can be adapted according to the specific requirements of the protein, including its solubility, stability and structure, and a detailed approach to the selection of expression system components and culture conditions affecting the optimisation of protein production and purification.

Summary of the Invention

The aim of the invention was to propose a new alternative recombinant hybrid protein useful in particular as a bioink component and as a biomaterial. Such a protein should be versatile, safe and effective for use both in the laboratory environment and ultimately in the patient. The aim of the invention was also to overcome the technical problems found in the state of the art, in particular by providing a protein with at least one parameter such as influence on cell viability, proliferation, adhesion or cytotoxicity (also in combination as a component of blends), rheological parameters (complex modulus, dynamic viscosity), printability (ability to be printed, resolution, fibre continuity and stability), mechanical properties (compressive mechanical strength, Young's modulus) comparable to or better than those currently used in the state of the art.

In the first aspect, the object of the invention is a recombinant hybrid protein REC2z sequentially arranged of four resilin domains, one 3xK+ domain, four resilin domains, one 3xK+ domain, three elastin domains, one K+ domain, four elastin domains and thirteen collagen domains. Preferably, the amino acid sequence of the REC2z protein contains 16% proline and/or 44% glycine. Preferably, the molecular weight of the protein is between 17 and 23 kDa, especially 18 kDa. Preferably, the amino acid sequence of each resilin domain is selected, independently of the other resilin domains, from the sequences SDTYGAPGGGNGGRP, GGRPSDSYGAPGGGN, GGRPSDSFGAPGGGN, GGRPSDSMGAPGGGN, PGGGNGGRPSDTYGA, GGRPSSSYGAPGQGN, GGRPSDSFGAPGGGN, GAPAQTPSSQY, AQTPSSQYGAP, and preferably is the sequence GGRPSDSYGAPGGGN. Preferably, the amino acid sequence of each elastin domain is selected, independently of the other elastin domains, from the sequence VPGXG, where X stands for V, I or A, and the sequence JPGZG, where J and Z independently stand for V, L or A, where, preferably, the amino acid sequence of each elastin domain is the sequence VPGAG. Preferably, the amino acid sequence of each collagen domain, independently of the other collagen domains, has the structure X-X'-G, where X stands for any amino acid, preferably proline, X' stands for any amino acid, preferably hydroxyproline, and G stands for glycine. Preferably, the amino acid sequence of each K+ crosslinking domain, independently of the other K+ domains, is the sequence GGKGGKGGKGG. Preferably, recombinant hybrid protein REC2z having the amino acid sequence of SEQ #2.

In the second aspect, the object of the invention is a recombinant DNA sequence selected from:

recombinant DNA sequence encoding the recombinant hybrid protein REC2z as defined above,

recombinant DNA sequence containing a region containing the DNA sequence encoding the recombinant hybrid protein REC2z as defined above, and

recombinant DNA sequence hybridising to the DNA sequence encoding the recombinant hybrid protein REC2z as defined above.

Preferably, the recombinant DNA sequence according to the invention has a sequence at least 80% identical, preferably at least 90% identical, and most preferably homologous with the recombinant DNA sequence of SEQ #1.

In a third aspect, the object of the invention is an expression vector comprising a recombinant DNA sequence comprising a region containing the recombinant DNA sequence as defined above and/or a recombinant sequence comprising a portion of a region containing the recombinant DNA sequence as defined above. Preferably, the expression vector according to the invention is a plasmid containing a promoter derived from the T7 phage, preferably selected from pET11a-d, pET15b, pET19b, pET28a-c(+), pET21a-d(+), pET22b(+), pET23a-d (+), pEt25b(+), pET44a-c(+), pET46Ek/LIC), and most preferably is pET11a.

In a fourth aspect, the object of the invention is an expression system, selected from available prokaryotic and eukaryotic systems, transformed with a recombinant DNA sequence comprising a region containing the recombinant DNA sequence defined above and/or a recombinant sequence comprising a portion of a region containing the recombinant DNA sequence defined above, the expression system being preferably a prokaryotic expression system, in particular *Escherichia coli*.

In the fifth aspect, the object of the invention is the use of the recombinant hybrid protein REC2z defined above as a component of a bioink for bioprinting.

In a sixth aspect, the object of the invention is the use of the recombinant hybrid protein REC2z defined above as a component of a biomaterial.

In a seventh aspect, the object of the invention is the use of a recombinant DNA sequence as defined above, or an expression vector as defined above, or an expression system as defined above to produce the recombinant hybrid protein REC2z as defined above.

The functional domains present in the recombinant hybrid protein REC2z, in particular the lysine-rich K+ crosslinking domains, enable the functionalisation of peptides with selected chemical groups, so that biomaterials enriched with recombinant hybrid proteins have a beneficial effect on facilitating the control of crosslinking in the bioprinting process.

The enrichment of the sequences of recombinant hybrid proteins with functional domains, increases their flexibility and enables the design of sites for possible chemical modifications, preferably altering the hydrophilic-hydrophobic nature of the resulting protein molecule.

Brief description of the figures

The invention in embodiments is illustrated in the drawing, wherein:

Fig. 1 shows a schematic of the recombinant structural protein REC2z;

Fig. 2 shows primer sequences for cloning and sequencing of genes encoding the recombinant hybrid protein REC2z;

Fig. 3 shows the DNA sequence of SEQ #1 encoding (expression cassette) the recombinant hybrid protein REC2z;

Fig. 4 shows the amino acid sequence of SEQ #2 of the recombinant hybrid protein REC2z;

Fig. 5 shows a map of the expression vector with the cloned gene culturing the hybrid protein REC2z;

Fig. 6A, 6B and 6C show SDS-PAGE separation images of samples taken from successive steps in the preparation of the recombinant hybrid protein REC2z (18.3 kDa);

Fig. 7 shows the level of fluorescence in alamarBlue staining of L929 cells on plates coated with REC2z protein compared to fibronectin in the L929 cell proliferation assay;

Fig. 8 shows the level of fluorescence in L929 cell cultures on plates coated with REC2z protein compared to fibronectin in the L929 cell adhesion test;

Fig. 9 shows the level of fluorescence in cultures of L929 cells on plates coated with REC2z protein compared to fibronectin in the cytotoxicity test of REC2z protein against L929 cells;

Fig. 10 shows the temperature dependence of the composite modulus - phase transition point;

Fig. 11 shows the results of the test of the viscosity of biomaterials in a temperature gradient;

Fig. 12 shows the dependence of storage modulus and loss modulus on deformation;

Fig. 13 shows the dependence of storage modulus and loss modulus on deformation for a test sample subjected to crosslinking for 20 s;

Fig. 14 shows the dependence of storage modulus and loss modulus on deformation for a test sample subjected to crosslinking for 30 s.

EXAMPLES

Example 1

The recombinant hybrid protein REC2z is sequentially arranged of four resilin domains, one 3xK+ domain, four resilin domains, one 3xK+ domain, three elastin domains, one K+ domain, four elastin domains, thirteen collagen domains (grouped into minor blocks of collagen 1x, 5x, 1x, 5x and 1x). Fig.1 shows a schematic of the recombinant hybrid protein REC2z, Fig. 3 shows DNA sequence SEQ #1 encoding (expression cassette) the recombinant hybrid protein REC2z, Fig. 4 shows the amino acid sequence of SEQ #2 of the recombinant hybrid protein REC2z. According

to this embodiment, the amino acid sequence of the recombinant hybrid protein REC2z contains 16% proline and 44% glycine, and the molecular weight of the protein is approximately 18 kDa.

Example 2

A plasmid vector containing a promoter derived from the T7 phage, namely pET11a, was used to obtain the recombinant hybrid protein REC2z according to the invention. Sequences encoding the recombinant hybrid protein REC2z were incorporated into the vector, namely DNA sequence SEQ #1 (Fig. 4) using molecular cloning methods known in the state of the art, with the selected restriction sites.

In particular, the restrictive sites were NdeI and BamHI. The cloned sequences were duplicated using selected primers with the sequences shown in Fig. 2. An expression vector allowing efficient and stable expression of recombinant proteins in *E. coli* cells from strain BLR(DE3) was obtained.

A map of the expression vector with the cloned gene encoding the hybrid protein REC2z is shown in Fig. 5.

Example 3

The recombinant hybrid protein RE2Cz was obtained by a method involving the following steps:

1. Bacterial cell culture of *E. coli* strain BLR(DE3) [optionally BL21(DE3) or a production *E.coli* strain] transformed with a plasmid encoding a recombinant hybrid protein with the addition of a suitable antibiotic (preferably ampicillin 50-200 μg/ml). The method uses conventional culture media, selected according to the host strain used. For the BL21(DE3) and BLR(DE3) strains used, this could be a standard, rich LB medium, further supplemented with proline and glycine. The cultures in the bioreactors use a mineral medium, the composition of which was originally developed during the experiments leading to the invention. The composition of the medium is shown below in Table 1.

Table 1: Composition of the mineral medium:

Enriched mineral substrate:	
$MgSO_4 \cdot 7H_2O$	0.20 g/L
K_2HPO_4	10.0 g/L
$NaHNH_4PO_4 \cdot 4H_2O$	4 g/L
Acetic acid	0.16 g/L
$CaCl_2 \cdot 2H_2O$	0.04 g/L
Glucose	0.2%
Trace salts	3 ml/L
Composition of trace salts:	
Iron (III) ammonium citrate	0.15 g/L
$MnSO_4 \cdot H_2O$	1.00 g/L
$ZnSO_4 \cdot 7H_2O$	2.78 g/L
$CoCl_2 \cdot 6H_2O$	1.86 g/L

$Na_2MoO_4 \cdot 2H_2O$	2.00 g/L
$CaCl_2 \cdot 6H_2O$	4.50 g/L
$CuSO_4 \cdot 5H_2O$	1.85 g/L
H_3BO_3	0.50 g/L
HCl	3.2%
Amino acids and aromatic compounds:	
Proline	0.2-0.8 mg/mL
Glycine	0.5-1.0 mg/mL

The advantage of using this mineral media is that it is relatively inexpensive, particularly for large-scale bioreactor-based cultures, and provides satisfactory growth rates (comparable to those of the media used in laboratory-scale cultures). In the first phase of culture, bacterial biomass is produced; the culture parameters for this phase are in the temperature range: 30 - 37 °C, stirring 150-700 rpm, air flow rate 3-10 LPM, DO (*dissolved oxygen*, i.e. the amount of oxygen dissolved in the liquid) >20%, pH 7.1 +/-1. The culture is growing until an optical density (OD_{600}) of 0.7 - 5.5 is obtained. Expression of the recombinant hybrid proteins is then induced by the addition of isopropyl-β-D-1-thiogalactopyranoside (IPTG) to a concentration of 0.4-1.0 mM or lactose to a concentration of 5-20 mM and culture is continued under the following conditions: temperature 37 °C, stirring 200-700 rpm, air flow rate 2-5 LPM, DO >20%, pH 7.1 +/-1 for 4-7 hours until an OD_{600} of 3.0-9.0 is reached.

2. At this stage, the bacterial biomass is separated from the culture medium by centrifugation. The bacterial cells are then resuspended in a lysis buffer with a composition developed for this purpose, shown in Table 2 below.

Table 2: Composition of the lysis buffer

NaCl	0.5 M
Tris HCl pH 7.5	50 mM
EDTA pH 8.0	10 mM
ß -mercaptoethanol	5 mM
Lysozyme	0.35 mg/ml
PMSF	1%
Benzamidine	10 mM

Disintegration of the bacterial biomass takes place using a high-pressure flow disintegrator with a set pressure of 800-900 bar. In the course of the work, it is preferable to perform 3 to 4 rounds of disintegration and add 0.4% (w/v) polyethyleneimine (PEI) to the suspension in order to precipitate host DNA.

3. Separation of insoluble protein fractions, non-disintegrated bacterial cells and precipitated DNA from the supernatant containing recombinant hybrid proteins by centrifugation.

4. Incubation of the supernatant containing the recombinant hybrid proteins at 90 °C for 15-30 min and centrifugation of the denatured proteins. During the course of the experiments, it was

also shown to be beneficial to add, at this stage, to the solution of recombinant proteins, a protease inhibitor cocktail (cOmplete, EDTA-free, Roche, cat. no. 05056489001) at a rate of 1 tablet per 50 ml of solution.

5. Precipitation of recombinant protein from solution by addition of ammonium sulphate to 30-50% saturation at room temperature, followed by centrifugation of the precipitated proteins and dissolution thereof in 20-40 mM TRIS buffer or phosphate buffer with 10 mM EDTA pH 8.0.
6. Dialysis of the recombinant protein suspension into 20-40 mM TRIS or phosphate buffer with 10 mM EDTA pH 8.0 for 24h-48h, at 4 °C.
7. Protein purification on *DEAE Sepharose Fast Flow* (GE Healthcare) bed or *Macro-Prep High Q Media* (Bio-Rad) bed or - alternatively - their equivalents. The bed-filled column was equilibrated with a calibration buffer composed of: 20-50 mM phosphate or TRIS buffer at pH 8.0. A protein solution obtained by dissolving the salted protein was applied to the column thus equilibrated. Separation was carried out by FPLC (Fast Protein Liquid Chromatography). Proteins not bound to the bed were eluted with the calibration buffer. Elution of proteins bound to the bed was performed with an elution buffer composed of 20-50 mM phosphate or TRIS buffer at pH 8.0 + 1 M NaCl. A flow rate of 1-2 ml/min. was used during separation and fractions above an absorbance of 0.05 AU were collected. The concentration of eluted protein was determined using the Bradford and BCA (Pierce BCA protein Assay Kit) method. According to the invention, the recombinant hybrid proteins REC2z did not bind to the bed and were eluted from the column with the calibration buffer.
8. Endotoxin removal using Pierce High-Capacity Endotoxin Removal Resin columns according to the manufacturer's instructions.
9. Dialysis of the recombinant protein suspension to ddH_20 for 24h-48h at 4 °C.
10. Lyophilisation of recombinant hybrid protein.

The yield of the described method is 20-60 mg of recombinant protein REC2z from 1 litre of culture. The course of the purification process for the recombinant structural protein REC2z in terms of product profile relative to impurities in SDS-PAGE electrophoresis under denaturing conditions is illustrated in Figs. 6A, 6B and 6C, showing images of SDS-PAGE separation of samples taken from successive steps to obtain the recombinant hybrid protein REC2z (18.3 kDa).

In the separation image shown in Fig. 6A: 1 - LMW protein mass standard; 2- *E. coli* BLR(DE3) /REC2z culture before induction; 3 - 1 h culture after IPTG induction; 4, 5, 6, 7, 8 - 2, 3, 4, 5 and 6 h culture after induction, respectively. The arrow marks the band corresponding to REC2z.

In the separation image shown in Fig. 6B: 1 - culture of *E. coli* BLR(DE3)/REC2z before induction; 2 - culture of BLR(DE3)/REC2z after induction; 3 - Broad Multi Color protein mass standard; 4 - supernatant after sonication; 5 - sediment after sonication; 6 - supernatant after sonication and incubation at 90°C; 7 - precipitate after sonication and incubation at 90°C; 8 - supernatant after sonication, incubation and desalting with ammonium sulphate at 0-30% saturation; 9 -

precipitate after sonication, incubation and ammonium sulphate desalting at 0-30% saturation; 10 - supernatant after sonication, incubation and desalting with ammonium sulphate at 30-50% saturation; 11 - precipitate after sonication, incubation and desalting with ammonium sulphate at 30-50% saturation; 12 - supernatant after dialysis I; 13 - precipitate after dialysis I.

In the separation image shown in Fig. 6C: 1 - Broad Multi Color protein mass standard; 2 - DEAE Sepharose Fast Flow bed entry; 3 - fractions unbound to DEAE Sepharose bed; 4 - fractions bound to DEAE Sepharose bed; 5 - Broad Multi Color protein mass standard; 6 - fractions entering Pierce High-Capacity Endotoxin Removal Resin bed; 7 - fractions eluted from High-Capacity Endotoxin Removal Resin bed; 8,9,10 - final protein solution intended for lyophilisation.

Example 4 – testing the biological activity of REC2z protein

The biological activity of the recombinant hybrid protein REC2z , including its effects on proliferation, adhesion and cytotoxicity, was tested. The cell lines used in the test were L929 - mouse fibroblasts, adherent cells growing in monolayer, ATCC cat. no. CCL-1. Reagents and apparatus routinely used in this type of test were used.

Composition of the culture medium

DMEM (*Dulbecco's Modified Eagle's Medium*) supplemented with 10% FBS, 4 mM L-glutamine, 4.5 g/L glucose,1mM sodium pyruvate, 1500 mg/L sodium carbonate, and 50 I.U./ml penicillin and 50 µg/ml streptomycin.

AlamarBlue reagent

AlamarBlue™ Cell Viability Reagent (Invitrogen, cat. no. DAL1100); resazurin, active ingredient of the reagent, non-toxic blue compound; when penetrated into living cells in a reducing environment, the conversion of resazurin to resorufin, which is red and highly fluorescent, is induced. An indicator of the number of live, metabolically active cells.

Cell proliferation

Obtained in culture of *E.coli* strain BLR DE3 and purified REC2z protein after lyophilisation was used to coat 96-position plates designed to culture eukaryotic cells in suspension (unmodified surface). An aqueous solution of REC2z protein at 1 and 5 µg/cm^3 was applied to the plates and dried. The control in the test was fibronectin used for coating at 1 µg/cm^3. Negative controls were wells not coated with protein. On the plates prepared in this way, cells of line L929 were seeded at 5x10^3/well. Cells were incubated in dedicated culture medium for 2, 24 and 48 hours. After this time, the culture wells were washed with sterile Phosphate-Buffered Saline solution (PBS buffer) to remove dead and non-adherent cells, the cells were stained with AlamarBlue reagent for three hours and fluorescence was measured at light wavelengths of 530 nm (excitation) and 590 nm (emission).

Increase in fluorescence after 48 hours relative to 2 and 24 hours taking into account the division time for cell line L929 (Fig. 7) indicates active cell proliferation in both the positive control and in

wells coated with the tested protein. It can therefore be concluded that the REC2z protein does not interfere with the rate of cell proliferation of the cell line used in the test.

Cell adhesion

Obtained in culture of *E.coli* strain BLR DE3 and purified REC2z protein after lyophilisation was used to coat 96-position plates designed to culture eukaryotic cells in suspension (unmodified surface). An aqueous solution of REC2z protein at 1 and 5 μg/cm^3 was applied to the plates and dried. The control in the test was fibronectin used for coating at 1 μg/cm^3. The negative control was the absence of any protein coating the well. Cells of line L929 at 1x10^4/well were seeded onto the plates prepared in this way. Cells were incubated in dedicated culture medium for 2, 4 and 24 hours. After this time, the culture wells were washed with sterile Phosphate-Buffered Saline solution (PBS buffer) to remove dead and non-adherent cells. In this case, washing was intended to remove non-adherent cells. Cells were stained with AlamarBlue reagent for three hours and fluorescence was measured at light wavelengths of 530 nm (excitation) and 590 nm (emission).

Increase in fluorescence during incubation (Fig. 8) indicates cell adhesion to the plastic used, both in the positive control and in wells coated with the tested protein. It was observed that the REC2z protein did not promote cell adhesion of the cell line used in the test to a degree comparable to fibronectin, a commercially available protein used to coat surfaces intended for eukaryotic cell culture.

Cytotoxicity

The cytotoxicity of the RE15mR protein against cells of the L-929 line (measurement of absorbance in the MTT assay) was tested according to ISO 10993-5:2009(E): *Biological evaluation of medical products. Part 5: In vitro cytotoxicity testing.* Depending on the planned exposure time, cells were seeded into 96-position plates at densities of 1x10^5/ml, 2.5x10^4/ml and 5x10^4/ml, respectively, at 100 μl per well.

For 24 hours, culture was conducted under standard conditions (5% CO_2 and 37 °C) in supplemented DMEM medium so that the fibroblasts had a chance to adhere to the bottom of the culture vessel. The test was performed using a direct method by adding, to the culture, a solution of purified protein reconstituted in medium. After controlling for confluence and population status, a protein solution in medium in the concentration range of 0.1 - 2 mg/ml was applied to the plates at 100 μl. A plate with cells at a density of 1x10^5/ml was incubated for 24 hours, while cultures at an initial density of 5x10^4/ml and 2.5x10^4/ml were exposed to REC2z protein for 48 and 72 hours, respectively.

After this time, the cells were incubated for 2 hours with the MTT reagent solution, and then all fluid from above the cells was removed and the resulting formazan salt crystals were dissolved with DMSO. The amount of colour product formed proportional to the number of living cells was determined by measuring absorbance at light wavelengths of 570 and 650 nm. According to the

standard, the expected result is a viability of cells exposed to the cytotoxic agent of not less than 70% of that of cells treated with nothing in the negative control (pink line on the attached graph).

Based on the results, REC2z protein in the concentration range (0.1 - 0.5 mg/ml) with 24-72 hour exposure was found not to be cytotoxic against the L-929 fibroblast line (Fig. 9).

Example 5 – testing of rheological properties

The obtained REC2z protein according to the invention was subjected to rheological parameter analysis. Measurements were taken at the point of gelation, viscosity was determined over a temperature gradient of 10-50°C, and oscillation levels were measured in non-crosslinking material at 20°C and in crosslinked material (30s 365 nm 13 mW/cm^2).

The following reagents were used in the test: HAMA (methacrylated hyaluronic acid) at 600.9 mg, LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate) as photoinitiator at 150.9 mg, REC2z recombinant protein [lot 2.Fer1.H001] at 3.3 mg, REC2z recombinant protein [lot 2.Fer2.E2] at 6 mg, PBSx1 [catalogue no: P4417-50TAB] – 30 ml was used from 1 tablet, NaOH – 60 µl of 5M solution, HCl – 10 µl of 5M solution.

Preparation of materials

The following biomaterials were used in the experiment:

HAMA 2% (w/v) + LAP 0.5% (w/v) solution [H2/L05]

30 ml of PBSx1 was transferred into a 50 ml falcon tube. Using an analytical balance, LAP was weighed out on a weighing dish and then quantitatively transferred 150.9 mg into a falcon tube with PBSx1. The falcon tube was wrapped in aluminium foil, transferred to a thermoblock and shaken at 800 rpm and 50°C. After dissolving the photoinitiator, the falcon tube was transferred to the refrigerator. Portions of the lyophilised HAMA were then weighed and transferred to a falcon tube with 600.9 mg photoinitiator solution. The HAMA solution was shaken in a thermoblock at 1000 rpm and 8°C, for 1 h. The final pH of the solution was 3.73. To adjust the pH, 60 µl of 5 M NaOH and 10 µl of 5M HCl were added, respectively, and the final pH was 7.21. The solution was then filtered using 0.22 µm syringe filters. The filtered solution was transferred sterile into an aluminium foil-wrapped 50 ml falcon tube and stored in the refrigerator.

HAMA solution 2% (w/v) + LAP 0.5% (w/v) + REC2z (2.Fer1.H001) [H2/L05/REC2z]

The lyophilised REC2z(2.Fer1.H001) protein contained in 10 glass vials was dissolved in 3.3 ml of a 2%HAMA solution with LAP by quantitative transfer of the solution 'from vial to vial'. Finally, taking into account losses on the walls of the vials, 2.4 ml of the mixture was obtained and used for further analyses.

HAMA solution 2% (w/v) + LAP 0.5% (w/v) + REC2z (2.Fer2.E2) [H2/L05/REC2z]

The lyophilised REC2z(2.Fer1.H001) protein contained in 3 glass vials was dissolved in 6 ml of a 2%HAMA solution with LAP by quantitative transfer of the solution 'from vial to vial'. Finally, taking

into account losses on the walls of the vials, 5.4 ml of the mixture was obtained and used for further analyses.

Rheological measurements

Rheological measurements were performed with an MRC 72 (Anton Paar) using the geometry of the PP25/S measuring system and the S-LP50/AL/S/G1 sandblasted table. The following dependencies were used to determine the rheological parameters:

- dependence of the composite modulus on the temperature gradient in a range of 40-0°C, with a frequency of 1 Hz and an amplitude of 30%;
- dependence of the composite modulus on shear stress and deformation; measurements were carried out both for non-crosslinked (0 s) and crosslinked samples for 20 s and 30 s using a UV-Vis lamp (Polbionica) with 365 nm wavelength and 13.0 mW/cm^2 power; the table was cooled to T=20°C before measurement; measurements were carried out with the following measurement parameters: frequency 1 Hz, amplitude 1-100%, T=20°C;
- variation of viscosity values in a temperature gradient of 10-40°C; stabilisation of the system prior to measurement was performed at T=10°C for 3 min at a shear rate of 10/s; viscosity measurements were carried out in a temperature gradient of 10-40°C at a shear rate of 50/s;

The detailed measurement conditions are shown in Table 3 below:

Table 3: Parameters for measuring rheological properties:

Parameter	Gelling point	Composite module	Viscosity
Temperature [^{0}C]:	40-0	20	I:10/II:10-40
Shear rate [1/s]	-	-	I:10/II:50
Frequency [Hz]	1	1	-
Amplitude [%]	30	1-100	-
Spindle [-]	PP25/S		
GAP [mm]	1.0		
Sample volume [ml]	0.65		

Results

The results of the analysis are shown in Table 4 below.

Table 4: Results of the analysis of the rheological properties

The analysis carried out	REFERENCE SAMPLE (2%HAMA_0.5%LAP)	SAMPLE TESTED (2%HAMA_0.5%LAP_1mg/mlREC2z)
Gelling point	Average module values: G'=26.32 Pa (±4.65) G"=82.89 Pa(±8.75)	Average module values: G'=25.12 Pa (±4.38) G"=84.59 Pa (±9.17)
Temperature gradient viscosity 10-50°C	η_{max}=176.99 mPas at T=12.07°C η_{min}=11.4 mPas at T=35.37°C	η_{max}=234.6 mPas at T=14.18°C η_{min}=26.526 mPas at T=35.91°C
Oscillations at 20°C non-crosslinked material	G'=0.0018-60.864 Pa G"=36.95-147.23 Pa	G'=4.4955-127.03 Pa G"=31.615-174.69 Pa
Oscillations at 20°C Cross-linked material 30s 365 nm 13 mW/cm2	G'=231.07-2888.4 Pa G"=80.521-959.71 Pa	G'=269.77-2339.5 Pa G"=172.41-915.49 Pa

The tests used a 2%HAMA(T46) solution with 0.5%LAP and 3.3mg of REC2z protein. For the analyses, a protein solution of 1mg/ml was prepared and juxtaposed with the reference material 2%HAMA0.5%LAP.

Gelling point

Fig. 10 shows the dependence of the storage modulus and the loss modulus in the temperature gradient for the materials tested (1 iteration).

No gelling point was observed for the analysed REC2z protein solution in the temperature range 0-40°C. Thus, it can be concluded that the absence of phase transitions in the tested temperature range indicates high thermal stability and thus may have a positive impact on the bioprinting process using the extrusion method, where temperature fluctuations adversely affect the process.

Viscosity analysis

Fig. 11 shows the results of a biomaterial viscosity test in a temperature gradient.

Table 5 below summarises the maximum and minimum viscosity values obtained from the analysis.

Table 5: Maximum and minimum viscosity values of the tested materials obtained from temperature gradient viscosity analysis

Designation	HAMA		HAMA_REC2z_2FER1.H001		HAMA_REC2z_2FER2.E2	
Repetition	η_{min}[mPas]	η_{max}[mPas]	η_{min}[mPas]	η_{max}[mPas]	η_{min}[mPas]	η_{max}[mPas]
I	7,3 (40,05°C)	176,99 (12,07°C)	26,5 (35,91°C)	**234,6** (14,18°C)	22,4 (28,67°C)	163,05 (12,07°C)
II	46,4 (40,05°C)	180,63 (24,53°C)			90,985 (40,05°C)	260,55 (10,99°C)

A viscosity test in a temperature gradient of 10-50°C indicates an increased viscosity of the REC2z protein solution compared to the reference material: η_{max}=234.6 mPas at T=14.18°C, η_{min}=26.526 mPas at T=35.91°C. The increased viscosity may improve the spatial stability of the structures just after extrusion, reducing the risk of layer spillage and increasing the precision of shape reproduction.

Testing the level of oscillations in non-crosslinked and crosslinked material [composite module]

The composite modulus describes the elastic and viscous properties of the biomaterial, i.e. the response of the components of the composite modulus through the resulting stresses to the forced oscillatory deformations. The composite modulus consists of the storage modulus G', which corresponds to elastic properties during sinusoidal deformation (represents stresses proportional to deformation – characteristic of elastic forces), and the loss modulus G'', which corresponds to viscous properties (represents stresses proportional to shear rate – characteristic of viscous forces).

Fig. 12 shows the dependence of the storage modulus G' and loss modulus G" on the deformation for the tested materials.

Table 6 below summarises the ranges of variation of the storage and loss moduli for the materials tested.

Table 6: The ranges of variation of the storage and loss moduli for the tested materials not exposed to 365 nm light.

Designation	HAMA		HAMA_REC2z_2FER1.H001		HAMA_REC2z_2FER2.E2	
Repetition	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]
I	0.0018-60.86	36.95-147.23	4.50-127.03	31.62-174.69	1.34-354.39	32.05-393.68
II	2.17-601.09	31.26-595.11			0.0017-1349.70	34.01-1422.60
III	0.0016-1305.40	31.25-905.86				

The non-crosslinked material shows higher elasticity than the reference sample.

Fig. 13 and 14 show the dependence of storage and loss moduli on deformation for the materials tested when exposed to 365 nm light for 20 s and 30 s, respectively.

Table 7 below summarises the ranges of variation of the storage and loss moduli for the materials tested.

Table 7: Ranges of variation of the storage and loss moduli for the materials tested that were cross-linked for 20s with 365 nm light.

Designation	HAMA		HAMA_REC2z_2FER1.H001		HAMA_REC2z_2FER2.E2	
Repetition	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]
I	140.88-844.68	63.845-501.74			192.5-894.2	90.36-567.67
II	161.65-1832.5	91.253-776.37			120.68-772.46	146.34-434.49

Table 8 below summarises the ranges of variation of the storage and loss moduli for the materials tested.

Table 8: The ranges of variation of the storage and loss moduli for the materials tested that were cross-linked for 30s with 365 nm light.

Designation	HAMA		HAMA_REC2z_2FER1.H001		HAMA_REC2z_2FER2.E2	
Repetition	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]	G' [Pa]	G" [Pa]
I	231-2888.4	80.521-959.71	269.77-2339.5	172.41-915.49	399.61-5377.7	249.31-1034.3
II	130.16-951.51	106.35-535.66			173.41-1811.8	129.27-1010.3

Testing the level of oscillation in the non-crosslinking material at 20°C yielded loss modulus (G") values in the range of 31.615-174.69 Pa. In the course of the tests, the analysed material achieved a value for low stress (G') more than 2,250 times higher and for high stress (G") more than 2 times higher than the reference sample analysed. It can therefore be concluded that the biomaterial obtained according to the invention exhibits a high elasticity even before crosslinking and can thus lead to shape retention during bio-printing especially in the case of complex geometries.

When the oscillation level of the UV-crosslinked material was tested at a wavelength of 365 nm for 30 s, a significant difference in G' values for low shear stress was observed, in the range of 172.41-915.49 Pa compared to the reference material. It can be concluded that the ability of rapid UV cross-linking allows for real-time layer fixation, which can be beneficial in multilayer bioprinting, particularly in applications requiring dynamic modulation of mechanical properties (e.g. tissue engineering).

Claims

1. A recombinant hybrid protein REC2z sequentially arranged of four resilin domains, one 3xK+ domain, four resilin domains, one 3xK+ domain, three elastin domains, one K+ domain, four elastin domains and thirteen collagen domains.

2. The recombinant hybrid protein REC2z according to claim 1, wherein the amino acid sequence comprises 16% proline and/or 44% glycine.

3. The recombinant hybrid protein REC2z according to claim 1 or 2, wherein the protein has a molecular weight of 17 to 23 kDa, preferably 18 kDa.

4. The recombinant hybrid protein REC2z according to any one of claims 1-3, wherein the amino acid sequence of each resilin domain is selected, independently of the other resilin domains, from among the sequences SDTYGAPGGGNGGRP, GGRPSDSYGAPGGGN, GGRPSDSFGAPGGGN, GGRPSDSMGAPGGGN, PGGGNGGRPSDTYGA, GGRPSSSYGAPGQGN, GGRPSDSFGAPGGGN, GAPAQTPSSQY, AQTPSSQYGAP, and preferably is the sequence GGRPSDSYGAPGGGN.

5. The recombinant hybrid protein REC2z according to any one of claims 1-4, wherein the amino acid sequence of each elastin domain is selected, independently of the other elastin domains, from the sequence VPGXG, where X stands for V, I or A, and the sequence JPGZG, where J and Z independently stand for V, L or A, wherein preferably the amino acid sequence of each elastin domain is the sequence VPGAG.

6. The recombinant hybrid protein REC2z according to any one of claims 1-5, wherein the amino acid sequence of each collagen domain, independently of the other collagen domains, has an X-X'-G structure, where X stands for any amino acid, preferably proline, X' stands for any amino acid, preferably hydroxyproline, and G stands for glycine.

7. The recombinant hybrid protein REC2z according to any one of claims 1-6, wherein the amino acid sequence of each K+ crosslinking domain, independently of the other K+ domains, is the sequence GGKGGKGGKGG.

8. The recombinant hybrid protein REC2z according to any one of claims 1-7, having the amino acid sequence of SEQ #2.

9. A recombinant DNA sequence selected from:

   a recombinant DNA sequence encoding the recombinant hybrid protein REC2z as defined in any one of claims 1-8,

   a recombinant DNA sequence containing a region containing a DNA sequence encoding the recombinant hybrid protein REC2z as defined in any one of claims 1 to 8, and

   a recombinant DNA sequence hybridising with a DNA sequence encoding the recombinant hybrid protein REC2z as defined in any one of claims 1-8.

10. The recombinant DNA sequence according to claim 9, having a sequence at least 80% identical, preferably at least 90% identical, and most preferably homologous to the recombinant DNA sequence of SEQ #1.

11. An expression vector comprising a recombinant DNA sequence containing a region containing the recombinant DNA sequence as defined in claim 9 or 10 and/or a recombinant sequence containing a portion of a region containing the recombinant DNA sequence as defined in claim 9 or 10.

12. The expression vector according to claim 11 being a plasmid comprising a promoter derived from phage T7, preferably selected from pET11a-d, pET15b, pET19b, pET28a-c(+), pET21a-d(+), pET22b(+), pET23a-d (+), pEt25b(+), pET44a-c(+), pET46Ek/LIC), and most preferably being pET11a.

13. An expression system, selected from a prokaryotic system and a eukaryotic system, transformed with a recombinant DNA sequence comprising a region containing the recombinant DNA sequence as defined in claim 9 or 10 and/or a recombinant sequence comprising a portion of a region containing the recombinant DNA sequence as defined in claim 9 or 10, wherein the expression system is preferably a prokaryotic expression system, in particular *Escherichia coli*.

15. Use of the recombinant hybrid protein REC2z as defined in any one of claims 1-8 as a component of a bioink for bioprinting.

16. Use of the recombinant hybrid protein REC2z as defined in one of claims 1-8 as a component of a biomaterial.

17. Use of the recombinant DNA sequence as defined in claim 9 or 10, or the expression vector as defined in claim 11 or 12, or the expression system as defined in claim 13 to produce the recombinant hybrid protein REC2z as defined in any one of claims 1-8.