Bioengineered collagens
Emerging directions for biomedical materials

John AM Ramshaw*, Jerome A Werkmeister, and Geoff J Dumsday
CSIRO Materials Science and Engineering; Clayton, Australia

Keywords: collagen, recombinant expression, triple helix, thermal stability, prokaryote, biomedical material, tissue engineering

Mammalian collagen has been widely used as a biomedical material. Nevertheless, there are still concerns about the variability between preparations, particularly with the possibility that the products may transmit animal-based diseases. Many groups have examined the possible application of bioengineered mammalian collagens. However, translating laboratory studies into large-scale manufacturing has often proved difficult, although certain yeast and plant systems seem effective. Production of full-length mammalian collagens, with the required secondary modification to give proline hydroxylation, has proved difficult in E. coli. However, recently, a new group of collagens, which have the characteristic triple helical structure of collagen, has been identified in bacteria. These proteins are stable without the need for hydroxyproline and are able to be produced and purified from E. coli in high yield. Initial studies indicate that they would be suitable for biomedical applications.

Recombinant Collagen: Establishing Commercial Availability

Collagen is the most abundant protein in mammals, where it plays critical roles in the structure and in molecular and cellular interactions in the extracellular matrix. For example, collagen is the major protein of many tissues, including skin, bone, ligament, tendon, and cartilage. Collagens are characterized by a unique molecular structure, the collagen triple-helix. This structure consists of a supercoiled triple-helix, which is made from three left-handed polyproline-like chains twisted together into a right-handed triple-helix. A further, interesting feature of this structure is that the tight packing of the triple helix requires that every third residue in the primary sequence be Gly, because there is no space for any larger amino acid in the interior axis of the triple-helix. As a consequence, a second characteristic feature of collagens is the repetitive amino acid sequence pattern (Gly-Xaa-Yaa). A further feature, is that the non-Gly positions are often occupied by the imino acid proline. For animal collagens, Pro residues in the Yaa position, about 10% of all residues, are normally posttranslationally modified by the enzyme prolyl-4-hydroxylase (P4H), to give 4-hydroxyproline (Hyp). This modification is very important for several reasons, particularly as an absolute requirement for good thermal stability of the triple-helix, but also to assist in self association, especially in the fibrillar collagens, and participation in certain receptor interactions. Although collagen is used as a single all-embracing term, in humans and other mammals, there are at least 28 genetically distinct types, all of which exemplify these various structural characteristics. The most abundant collagens are the interstitial, fibril forming collagens, particularly type I collagen, which are present in all the major connective tissues (Fig. 1). Type I collagen, which can be readily obtained and purified, has become the major collagen used in biomedical and tissue engineering applications.

Although there are some early examples, it is only in the past 100 years that a range of collagen-based biomedical materials have emerged, starting from the use of human amnion in 1912, followed by collagen based dressings in the 1940s, with many subsequent products. Most recently, there has been a growing interest in using natural extracellular matrix-based, particularly collagen, supporting structures (scaffolds) in tissue engineering developments.

In all these cases of medical applications, there are a number of concerns that have been expressed. These include, for example, the natural variability in preparations from animal tissues, where purity and predictability of performance are issues. Of more concern has been the possibility of transmission of animal based diseases, especially bovine spongiform encephalopathy (“mad-cow disease”). There have been two suggested approaches to this disease issue. One approach has been the examination of non-mammalian species, including jellyfish, fish, and chicken as material sources, while the other approach has been the production of collagen by recombinant technology. Recombinant approaches enable production of a disease-free product that is of uniform product quality. The approach also could allow for the production of less common collagen types, where extraction from tissue is not practical. There is also the opportunity to modify and improve on the natural protein structures by introduction of mutated residues, selection of specific domains, engineering products with multiple repetitive motifs, and development of chimeric structures.

The potential disadvantage of recombinant technology, however, is that recombinant expression of type I collagen, or any mammalian collagen, will almost certainly also need the co-expression of functional P4H to enhance the triple-helical stability. The P4H enzyme consists of two different chain types,
Each with a molecular mass of around 65 kDa. If the stability of the collagen triple-helix is low, then the product is the denatured form of collagen, gelatin. Gelatin consists of only single chains that are not folded into in the native triple-helical structure. Also, while recombinant technology can be used easily for small scale production of laboratory samples, for commercial scale production there will be manufacturing issues that will need to be solved and process economics evaluated.

Initial biotechnology developments focused on the production of full-length human collagens, including the heterotrimeric type I collagen and the homotrimeric type III collagen. Mammalian cell systems, such as HT1080 and HEK 293-EBNA cells, have been used, but yields are very low. The same is also true for production in an insect cell system, Baculovirus. Both systems, however, do have endogenous P4H activity (Table 1). Microorganisms provide alternative expression systems and are a natural choice because of their familiarity in the biotechnology industry. Unfortunately, the use of E. coli expression was initially not seen as practical, as there seemed to be difficulties in co-expressing all the necessary genes. On the other hand, yeast systems were considerably more promising, and several have been used successfully for the expression of full-length hydroxylated human collagens (Table 1). The advantage of the yeasts is they can be optimized to integrate and co-express 3 or 4 genes concurrently. The most successful application, which has provided commercial opportunities, has emerged from expression in P. pastoris (Table 1). Yeasts, as with other microorganisms typically require different codon preferences to those in mammalian systems for optimal expression. The development of rapid gene synthesis enables effective codon optimized sequences as well as specific variants of these sequences to be readily produced resulting in giving good yield.

Other approaches to improve commercial yields are also possible and include modification of the propeptide domains, enhanced integration, increased copy number of introduced genes, and reduced production temperature and oxygen enrichment to increase the P4H activity.

Other systems have also emerged, based on transgenic organisms. For collagens, the mammary gland has been used for collagen production in mouse milk (Table 1). Another, more unusual system that is suited for fibrous proteins has been production of recombinant human type III procollagen in cocoons of transgenic Bombyx mori silkworms. Collagen production in transgenic plants has also been developed and seems to provide cost-effective, commercial outcomes. Initial reports produced unhydroxylated or poorly hydroxylated collagen from leaves and from seeds. Additional introduction of the mammalian P4H is necessary for a quality collagen product, even though plants contain a form of P4H. Most recently, heterotrimeric collagen has been produced with P4H also present, giving excellent protein yields, which can be commercialized.

There may be further advantages of recombinant systems that possibly work only at a laboratory level. For example a range of constructs have been made for type II collagen, where specific “D-period” repeats, about 234 amino acids each, have been removed or substituted into the structure, allowing a better understanding of collagen structure and function. Another variation to this approach has been the design and production of small collagen fragments with embedded multiple repetitive domains (integrin) within the collagen chain.

Recombinant Bacterial Collagen: An Emerging System

Collagens were seen historically as being associated with multicellular animal tissues. In more recent times the suggestion of collagen-like molecules in other species has emerged, with (Gly-Xaa-Yaa)n repeating sequences present in a few fungi, in viruses, and in phage. Most interesting, however, are the numerous collagen-like structures that are being identified in bacteria and in the emerging, large numbers of bacterial genomes. Thus, a gene with a collagen-like repeat, that is a virulence factor, was identified and characterized in the bacterium Streptococcus pyogenes. Subsequently, a second, structurally distinct, collagen-like gene was identified from the same species. Both were shown by biochemical and biophysical studies to have the characteristic collagen triple-helical structure.

The surprise that emerged was that these collagens were both stable around mammalian body temperature, with melting temperatures of 36.4 °C and 37.6 °C, without the presence of any hydroxyproline.

A decade ago, Rasmussen and colleagues reported an analysis of the available bacterial genome databases, searching for collagen-related structural motifs (CSM). The study included 137 eubacterial genomes and 15 archaeabacterial genomes and looked for sequences with homology to (Gly-Pro-Pro), where n was 7 or more. This identified 53 proteins in 25 bacterial
genomes, with none of these in any of the 15 archaebacterial genomes. Several of the bacterial genomes contained several CSM, up to 9 in some cases in this analysis. These motifs showed a wide range in size, with a mean length of 76 Gly-Xaa-Yaa triplets, and the collagen-like sequences are always flanked by non-collagenous domains. Although all show collagen like sequence motifs, it is very unlikely that they will all contain a stable collagen triple-helix, especially for those where as few as only 7 repeats are present. It has been suggested that a larger number of repeats, perhaps 35 or more, may be required for triple helix formation and stability. Also, while the database search has identified many putative sequences in various genomes, the evidence for natural expression is limited to relatively few. A more detailed analysis of amino acid compositions suggested different groups of collagen-like proteins. These were considered as a Thr-rich group, a Pro-rich group, and a group rich in charged residues. The collagen-like sequences from different bacteria all had a relatively high Pro content, generally in the Xaa position, rather than in the Yaa position which in animal collagens is then frequently found as Hyp. In contrast, in the bacterial sequences there is a higher proportion of threonine and glutamine in the Yaa position. The other interesting trend was the uneven distribution of charged residues; most frequently the X positions having a negative charge, the Y positions a positive charge. Subsequently, the extent of the genomic information has increased many-fold and a large number of additional genomes are available for study. While the initial motifs that were identified were typically from pathogens, a wide range of collagen-like motifs are also emerging from non-pathogenic species.

It was suggested that bacterial collagen sequences arise from horizontal gene transfer from eukaryotes to bacteria. However, whatever the genetic origins, it is clear that a wide range of structures, functions, and molecular interactions have evolved and become established. The few collagen-like structures from pathogenic species that have been well studied suggest that the collagen motif is typically associated with the outer membrane of the bacteria, and may interact with the host to assist invasion or to help a pathogen evade the host immune system. This binding can be, for example, to integrin receptors or to other extracellular matrix molecules, mediating pathogen internalization by human cells.

The identification of this new group of collagens provides potential for development of new, recombinant biomedical materials. Several have now been studied after expression in E. coli. They do not contain any hydroxyproline, and even though some native bacterial structures are glycosylated, this is not the case with the recombinant products. Also, although a large number of species contain a (Gly-Xaa-Yaa) sequence repeat, only eight have been examined in detail. In seven of these cases—two from S. pyogenes, and one from each of Bacillus anthracis, Clostridium perfringens, Solibacter usitatus, Rhodopsseudomonas palustris and Methylobacterium sp 4-46 (Fig. 2)—the characteristic CD spectrum of the collagen triple-helix, with a maximum near 220 nm and a minimum near 198 nm, has been observed, and a melting temperature, Tm, has been established.

| Production systems | Yield | Comments |
|--------------------|-------|----------|
| Mammalian cells:   |       |          |
| HT1080             | ≤1 mg/L | Mainly type II collagen and variants |
| HEK293             | ≤80 mg/L | Used to produce collagen types V, VII, VIII, X, XVI |
| CHO                | <0.5 mg/L | Type IV collagen |
| COS-1              | <0.5 mg/L | Type III and XVII collagens |
| Insect cells       |       |          |
| Baculovirus       | ≤40 mg/L | Best yield for homotrimer, type III collagen and less heterotrimer type I collagen |
| Bacterial cells    |       |          |
| E. coli           | ≤14 g/L | No hydroxylation |
| Yeast cells:       |       |          |
| S. cerevisiae     | ≤0.4% protein | Incomplete hydroxylation |
| P. pastoris       | ≤1.5 g/L | Fully hydroxylated. Commercially available |
| H. polymorpha     | ≤0.6 g/L | For a 14 kDa fragment. No hydroxylation |
| Transgenic animals |       |          |
| Mouse (milk)      | ≤8 g/L | For a 37kb fragment. Much lower yields for full-length constructs |
| Silkworm (Bombyx mori) (cocoon) | ≤4.2 mg per cocoon | Probably not triple-helical, but rather as gelatin |
| Transgenic plants |       |          |
| Plant seeds (Barley, Rice, Maize) | ≤140 mg/Kg of seed (barley) | For a 45 kDa fragment. Considerably less for full-length collagen. Low hydroxylation |
| Plant leaves (Tobacco) | ≤20 g/L of soluble protein | Yield can depend on hydroxylation status |

*Compiled from data presented previously.*
using CD with increasing temperature (Fig. 1), confirming the presence of the triple-helical structure. Additionally, for several of these structures and one further example, *Legionella pneumophila*, the presence of a collagen triple-helix has been inferred from protease resistance to enzymes including trypsin, chymotrypsin and pepsin. Of great interest was the fact that although the bacterial collagens expressed in *E. coli* do not contain Hyp, they are all, nevertheless remarkably stable (Fig. 2) with $T_m$'s in the range of $\sim 35-39 \, ^\circ\text{C}$, similar to $T_m \sim 37 \, ^\circ\text{C}$ for human collagens. The lack of Hyp residues may also account in part for bacterial collagens not forming fibrillar structures, unlike type I collagen (Fig. 1). For those studied, it seems that in most cases the presence of non-triple-helical N- or C-terminal domains had little effect on the stability of the triple-helix. The relatively high content of Pro residues in all of these proteins is an important stabilizing factor for the triple-helix structure. However, additionally, other stabilization strategies are in place. Some bacterial collagens, such as those from *S. pyogenes*, are rich in charged residues and stabilized by electrostatic interactions, while polar residues may contribute to the stability of some of the other bacterial collagens. It is interesting to speculate that the bacterial collagens from pathogenic species have high $T_m$ values that are adapted for attachment to host mammalian tissues, but this reasoning may not translate to non-pathogenic species, where a different environmental temperature may be important.

The high stability of bacterial collagens is clearly important for both the production and application of a bacterial collagen for biomedical applications. Other key features have also been assessed, particularly in relation to safety. Thus *S. pyogenes* Scl2 derived collagen, lacking any terminal domains, has been shown to be non-cytotoxic in cell culture (Fig. 3) using mouse L929 cells and human WI-38 and HT1080 cells, and to be non-immunogenic in both inbred and outbred mouse strains. There was a marginal response when adjuvant was used, but the response was much less than previously observed with avian collagen and bovine medical collagen.

A further positive for developing bacterial collagen as a new medical material is that it can be readily produced in good quantity. No doubt, this is helped in part by the bacterial collagens being shorter than the human fibrillar collagen sequences. For example, the *S. pyogenes* Scl2 collagen domain comprises 78 Gly-Xaa-Yaa triplets compared with the 338 triplets found in human collagen type I. To date, production studies have used a pColdIII (Takara Bio Inc) vector for expression in *E. coli*, as this vector system had proved useful for small scale expression of bacterial collagens from various species. However, it is possible that other vectors could give better commercial yields? Production in shake flask cultures gives low yields of recombinant product, <1 g/L, but significantly better yields are possible when production is transferred to fed batch stirred tank bioreactors, especially when a high cell density strategy is used. The best yields, of up to 19 g/L, were reported for a *S. pyogenes* Scl2 construct using a high cell density strategy and an extended 24 h production time. The construct contained an N-terminal non-collagenous domain, but this still gives a calculated yield of the collagen only domain of around 14 g/L. The observed protein yields

![Figure 2. A schematic illustrating the structures and melting temperatures, $T_m$, of bacterial collagens, including the non-triple-helical terminal domains (blue) and the central triple helical domain (red) for which a triple-helical structure has been established.](image)

![Figure 3. Fibroblasts growing on and within pores of a bacterial collagen sponge, stained after 7 d with a Live/Dead - Viability/Cytotoxicity Kit assay (Molecular Probes). Live cells are shown as green; any dead cells would appear red. Bar = 250 µm.](image)
A key biotechnology consideration is what products could be developed from the new bacterial collagen-based materials? There would certainly seem to be opportunities in biomedical products, as with mammalian collagens, either where the new collagens are used as coatings or fabricated into a specific device, to provide enhanced products for specific applications. For example, as a coating, bacterial collagen can be bioengineered to introduce discrete integrin-binding sequences, GLPGER, GFPGER, or GFPGEN, to target specific integrin domains (α1 I-domain or α2 I-domain). This provides coating substrates that can discriminate between endothelial cell and smooth muscle cell adhesion while avoiding any platelet aggregation, which would have specific applications for vascular prostheses and other cardiovascular technologies. The ability to produce bacterial collagens as designed bioactive hydrogels and in other formats, such as sponges, further extends the potential biomedical and tissue engineering applications. Recent studies have demonstrated that an adequate shelf life for this type of product can also be achieved when appropriate stabilization and dry storage strategies are used.

Conclusions and Future Trends

In addition to the present examples that show the promise of recombinant, bioengineered bacterial collagens in biomedical and tissue engineering applications, other exciting developments could emerge in future. These could include constructs where the intrinsic stability has been further enhanced. This could arise from the discovery of new, naturally more stable molecules, such as the high stability, Tm = 42 °C, collagen found in a bacteriophage collagen, or by modifications to the sequence that incorporate the existing understanding of how different sequence features, including electrostatic interactions, can enhance stability. The other option to increase stability is to add Hyp, which is currently not included in the E. coli production system. Previously, it has been suggested that this could be achieved by adding the modified amino acid Hyp to the fermentation medium, although the efficiency of incorporation may be limited and there would not be specificity to the Yaa position in the sequence. Recently, however, a system that allows Yaa-position specific post-translational hydroxylation of collagen domains during fermentation in E. coli has been described and shown to be effective for short, peptide length collagen substrates. The use of this approach for bacterial collagens may be limited by their larger size and the high proportion of Pro in the Xaa position. A further potential area for development is that of chimeric structures. These could include composite structures of different bacterial collagen components, for example, using different species for the folding and collagen domains, or different collagen domains, or constructs that include bacterial collagens and different proteins, which, for example, could be silks, elastic proteins, or other collagens. Opportunities also exist to further functionalize the bacterial collagen core protein to allow selective simple and complex tethering of one of more peptides or non-peptide molecules for off the shelf development of specific protein materials with a defined application.

Collagen has developed a well-established, and well-deserved, position as a useful material in biomedical devices, where a variety of different formats including manufactured
tissue and solubilized collagen formats have been developed. These products have been used successfully in many different clinical applications. However, in certain cases, such as with a manufactured tissue-based dura mater replacement, unfortunate adverse consequences have been observed, in this example leading to death due to disease transmission. This example, and other potential problems arising from disease transmission, mean that there is still a concern about using native collagen-based materials and devices. Notable progress has been made on commercial, recombinant production of human collagens. However, the rapidly emerging technologies that have characterized and produced recombinant non-animal (bacterial) collagens in E. coli\textsuperscript{23-27,28} have introduced an exciting new collagen option that can be readily customized for specific biomedical applications.\textsuperscript{28-33} These customized collagens will pave the way for more diverse applications in medical devices, implantable materials and potentially as scaffolds for stem cells, to name but a few examples of uses for these recombinant bacterial collagens.

To enable commercialization, future demonstration of the high fermentation yields and straightforward purification process at pilot-scale (e.g., 500 L batch size) is required to show scalability and evaluation of process economics. The pilot-scale batches will also facilitate production of large amounts of material that can then be used for more extensive biological evaluation and applications development in general animal models followed by defined functional animal models for specific biomedical applications.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.

Acknowledgments
We wish to thank Jacinta F White for the transmission electron micrograph and Aditya Vashi for the histology photograph. This work was supported in part through NIH grant #EB011620.

References
1. Brodsky B, Ramshaw JAM. The collagen triple-helix structure. Matrix Biol 1997; 15:545-54; PMID:9185287; http://dx.doi.org/10.1016/S0945-053X(97)90030-5.
2. Ricard-Blum S. The collagen family. Cold Spring HarbPerspect Biol 2011; 3:a004978; PMID:21423911; http://dx.doi.org/10.1101/cshperspect.a004978.
3. Ramshaw JAM, Werkmeister JA, Peters DE. Collagen as a biomaterial. In, Current Perspectives on Implantable Devices, Vol. 2. (Ed. Williams, D.F., JAI Press, London), 1990; 151-220.
4. Ramshaw JAM, Peng YY, Glattauer V, Werkmeister JA. Collagens as biomaterials. J Mater Sci Mater Med 2009; 20(Suppl 1):S3-8; PMID:18379838; http://dx.doi.org/10.1007/s10856-008-3415-4.
5. Badyak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater 2009; 5:1-13; PMID:18938117; http://dx.doi.org/10.1016/j.actbio.2008.09.013.
6. Peng YY, Glattauer V, Ramshaw JAM, Werkmeister JA. Evaluation of the immunogenicity and cell compatibility of avian collagen for biomedical applications. J Biomed Mater Res A 2010; 93:1235-44; PMID:20275233; http://dx.doi.org/10.1002/jbm.a.32905.
7. Werkmeister JA, Ramshaw JAM. Recombinant protein scaffolds for tissue engineering. Biomater Med Dev 2012; 7:012002; PMID:22262725; http://dx.doi.org/10.1088/1748-6041/7/1/012002.
8. Williams DJ, Sebstiane IM. Tissue engineering and regenerative medicine: manufacturing challenges. IEE Proc Nanobiotechnol 2005; 152:207-15; PMID:1651491.
9. Perret S, Miele C, Bernocco S, Berland P, Garrone R, Hulmes DJ, Theisen M, Ruggiero F. Unhydroxylated triple helical collagen I produced in transgenic plants provides new clues on the role of hydroxyproline in collagen folding and fibril formation. J Biol Chem 2001; 276:6356-63; PMID:11557756; http://dx.doi.org/10.1074/jbc.M105570200.
10. Eskelin K, Ritala A, Suntio T, Blumer S, Hölker H, Wahlström EH, Baez J, Mäkinen K, Maria NA. Production of a recombinant full-length collagen type I α1(I) and of a 45-kDa collagen type I α1(I) fragment in barley seeds. Plant Biotechnol J 2009; 7:657-72; PMID:19663532; http://dx.doi.org/10.1111/j.1747-6659.2009.00432.x.
11. Shoseyov O, Posen Y, Gryspan F. Human recombinant type I collagen produced in plants. Tissue Eng Part A 2013; 19:1527-37; PMID:23252967; http://dx.doi.org/10.1089/teng.2012.0347.
12. Majsterek I, McAdams E, Adachi E, Dhampe ST, Ferratala A. Prospects and limitations of the rational engineering of fibrillar collagens. Protein Sci 2003; 12:2063-72; PMID:12931084; http://dx.doi.org/10.1002/pro.835103.
13. Peng YY, Werkmeister JA, Vaughan PR, Ramshaw JAM. Constructs for the expression of repeating triple-helical protein domains. Biomater Med Dev 2009; 4:015006; PMID:19891541; http://dx.doi.org/10.1088/1748-6041/4/1/015006.
14. Wang C, St Leger RJ. A collagenous protective coat enables Metarhizium anisopliae to evade insect immune responses. Proc Natl Acad Sci U S A 2006; 103:6647-52; PMID:16614065; http://dx.doi.org/10.1073/pnas.0609151103.
30. Caswell CC, Oliver-Kozup H, Han R, Lukomska E, Lukomska S. Scfl, the multifunctional adhesion of group A Streptococcus, selectively binds cellular fibronectin and laminin, and mediates pathogen internalization by human cells. FEMS Microbiol Lett 2010; 303:61-8; PMID:2002194; http://dx.doi.org/10.1111/j.1574-6968.2009.01864.x

31. Han R, Zwieka A, Caswell CC, Xu Y, Keene DR, Lukomska E, Zhao Z, Hooik M, Lukomski S. Assessment of prokaryotic collagen-like sequences derived from streptococcal Scf1 and Scf2 proteins as a source of recombinant GXY polymers. Appl Microbiol Biotechnol 2006; 72:109-15; PMID:16552563; http://dx.doi.org/10.1007/s00253-006-0387-5

32. Yoshizumi A, Yu Z, Silva T, Thigagaraj G, Ramshaw JA, Inouye M, Brodsky B. Self-association of streptococcus pyogenes collagen-like constructs into higher order structures. Protein Sci 2009; 18:1241-51; PMID:19472339; http://dx.doi.org/10.1002/pro.154

33. Peng YY, Yoshizumi A, Danon SJ, Glattauer V, Proskopenko O, Mirochnitchenko O, Yu Z, Inouye M, Werkermeister JA, Brodsky B, et al. A Streptococcus pyogenes derived collagen-like core as a non-cytotoxic and non-immunogenic cross-linkable biomaterial. Biomaterials 2010; 31:2755-61; PMID:20056274; http://dx.doi.org/10.1016/j.biomaterials.2009.12.040

34. Peng YY, Howell L, Stoichevska V, Werkeister JA, Dumsday GJ, Ramshaw JAM. Towards scalable production of a collagen-like protein from Streptococcus pyogenes for biomedical applications. Microb Cell Fact 2012; 11:146; PMID:23126526; http://dx.doi.org/10.1186/1475-2859-11-146

35. Peng YY, Stoichevska V, Madsen S, Howell L, Dumsday GJ, Werkeister JA, Ramshaw JAM. A simple cost-effective method for large-scale purification of recombinant non-animal collagen-like proteins. Appl Microbiol Biotechnol 2014; 98:1807-15; PMID:23088839; http://dx.doi.org/10.1007/s00253-013-5475-8

36. Caswell CC, Barczyk M, Keene DR, Lukomska E, Guillberg DE, Lukomski S. Identification of the first prokaryotic collagen sequence motif that mediates binding to human collagen receptors, integrins α2β1 and α1β1. J Biol Chem 2008; 283:36068-75; PMID:18990704; http://dx.doi.org/10.1074/jbc.M806685200

37. Seo N, Russell BH, Rivera J, Liang X, Xu X, Afshar-Kharghan V, Hook M. An engineered α1 integrin-binding collagenous sequence. J Biol Chem 2010; 285:31046-54; PMID:20675378; http://dx.doi.org/10.1074/jbc.M110.151357

38. Peng YY, Stoichevska V, Schacht K, Werkeister JA, Ramshaw JAM. Engineering multiple biological functional motifs into a blank collagen-like protein template from Streptococcus pyogenes. J Biomed Mater Res 2014; http://dx.doi.org/10.1002/jbm.a.34989; Forthcoming 2014.

39. An B, DesRochers TM, Qin G, Xia X, Thigagaraj G, Brodsky B, Kaplan DL. The influence of specific binding of collagen-silk chimeras to silk biomaterials on hMSC behavior. Biomaterials 2013; 34:402-12; PMID:23088839; http://dx.doi.org/10.1016/j.biomaterials.2012.09.085

40. Yu Z, Visse R, Inouye M, Nagase H, Brodsky B. Defining requirements for collagenase cleavage in collagen type III using a bacterial collagen system. J Biol Chem 2012; 287:22988-97; PMID:22573319; http://dx.doi.org/10.1074/jbc.M111.348979

41. Sweeney SM, Orgel JP, Ferralda A, McAuliffe JD, Turner KR, Di Lullo GA, Chen S, Antipova O, Perumal S, Ala-Kokko L, et al. Candidate cell and matrix interaction domains on the collagen fibril, the predominant protein of vertebrates. J Biol Chem 2008; 283:21817-97; PMID:18487200; http://dx.doi.org/10.1074/jbc.M709319200

42. Bini E, Foo CW, Huang J, Karageorgiou V, Kirchel B, Kaplan DL. RGD-functionalized bioengineered spider dragline silk biomaterial. Biomacromolecules 2006; 7:3139-45; PMID:17096543; http://dx.doi.org/10.1021/bm0607877

43. Browning MB, Dempsey D, Guiza V, Becerra S, Rivera J, Russell B, Hook M, Chubb F, Miller M, Fossum T, et al. Multilayer vascular grafts based on collagen-mimetic proteins. Acta Biomater 2012; 8:1010-21; PMID:22142564; http://dx.doi.org/10.1016/j.actbio.2011.11.015

44. Cosgriff-Hernandez E, Hahn MS, Russell B, Wilems T, Munoz-Pinto D, Browning MB, Rivera J, Hook M. Bioactive hydrogels based on Designer Collagens. Acta Biomater 2010; 6:3969-77; PMID:20466808; http://dx.doi.org/10.1016/j.actbio.2010.05.002

45. Luong PT, Browning MB, Bixler RS, Cosgriff-Hernandez E. Drying and storage effects on poly(ethylene glycol) hydrogel mechanical properties and bioactivity. J Biomed Mater Res A 2013; http://dx.doi.org/10.1002/jbm.a.34977; In press; PMID:24137325.

46. Persikov AV, Ramshaw JA, Brodsky B. Prediction of collagen stability from amino acid sequence. J Biol Chem 2005; 280:19343-9; PMID:15753081; http://dx.doi.org/10.1074/jbc.M501657200

47. Buechter DD, Paulella DN, Leslie BS, Brown MS, Mehos KA, Gruskina EA. Co-translational incorporation of trans-4-hydroxypoline into recombinant proteins in bacteria. J Biol Chem 2003; 278:645-50; PMID:12999455; http://dx.doi.org/10.1074/jbc.M209364200

48. Pinkas DM, Ding S, Raines RT, Barron AE, Tunable, post-translational hydroxylation of collagen Domains in Escherichia coli. ACS Chem Biol 2011; 6:320-4; PMID:21210682; http://dx.doi.org/10.1021/cb100298c

49. Braccalelo A, Santopietro V, Vassalll M, Marletta G, Del Gaudio R, Bochicchio B, Pepe A. Design and production of a chimeric resilin-, elastin-, and collagen-like engineered polypeptide. Biomacromolecules 2011; 12:2957-65; PMID:21767089; http://dx.doi.org/10.1021/bm2005388

50. Brown P, Brandel JP, Sato T, Nakamura Y, MacKenzie J, Will RG, Ladogana A, Pocchiari M, Leschek EW, Schonberger LB. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 2012; 18:901-7; PMID:22607808; http://dx.doi.org/10.3201/eid1806.120116