Comparing Natural Collagen with biomimetic collagen

Natural collagen

1. **General properties of natural collagens**

Collagen is one of the most abundant proteins in nature. This is a major macromolecular component of the extracellular matrix and a scaffold of animal connective tissues. It comprises up to one third of all proteins in the human body mass. Three quarters of the dry weight of skin consists of collagen. Mechanical strength, thermal stability and ability to engage in multiple molecular interactions are essential features of this important biological molecule.

2. **Classification of different type of collagens**

Currently, 29 different types of collagen with 46 types of different polypeptide chains are known (Shoulders and Raines, 2009). A number of other protiens contain collagen-like sequences and domains.

There are four major categories of collagen: classical fibrillar collagens, FACITs (fibril-associated collagens with interrupted triple helices), MACITs (membrane-associated collagens with interrupted triple helices) and MULTIPLEXINs (multiple triple-helix domains and interruptions) (Shoulders and Raines, 2009). They differ in their tissue and organ distribution, as well as chemical composition. The polypeptide strands in triple helical collagen can be identical or different. Heterotrimeric triple helices are more common than homotrimeric versions. Heterotrimeric triple helices may consist of two or three different strands (AAB and ABC compositions).

3. **The common structural domain - triple helix**

Collagen consists of three left-handed parallel strands, known as alpha chains, in polyproline II type (PPII) helical conformation. These helices coil around each other to form a right-handed triple helix. PPII helices are tightly packed resulting in each third residue being a glycine. Repeating sequence motif XaaYaaGly is observed in all collagen types, although sometimes it is disrupted at some positions in nonfibrillar collagens. Xaa and Yaa can be any amino acids but very often Xaa is proline (Pro) and Yaa is 4-hydroxyproline (Hyp). Around 22% of all residues in human collagen are prolines or 4-hydroxyprolines. Triplet motif ProHypGly is the most common in collagen sequences.

Expression of collage genes results of the synthesis of approximately 300 nm long protocollagen polypeptide chains that are flanked by N-terminal and C-terminal propeptide sequences. This is a starting material for formation of building blocks and further macromolecular assembly of collagen microfibrils and fibers. Protocollagen undergoes posttranslational modification which involves introduction of hydroxyl groups with the help of prolyl-4-hydroxylase and lysyl hydroxylase, as well as disulfide bonds between C-terminal propeptides. Modified strands assemble into triple helices of procollagen (Shoulders and Raines, 2009).

Propeptides are essential for formation of triple helices but prevent the assembly of fibrils and therefore has to be removed. Procollagen N- and C-proteinases partially digest propeptides leaving shorter non-triple helical telopeptides. The tropocollagen triple helices formed as a result of this process are up to 300 nm long and 1-2 nm thick.

Once formed, individual triple helices of tropocollagen are able to assemble in a complex hierarchical manner leading to complex structures such as fibers and their networks observed in tissues.

It is interesting that tropocollagen is unstable at body temperature but the formation of fibrils has a stabilizing effect on the triple helices of tropocollagen. Telopeptides at the C- and N-termini of tropocollagen are essential for self-assembly of fibrils. It is proposed that they interact with specific binding sites on triple helical monomers to initiate the process. Synthetic telopeptides might be unable to recognize binding sites on tropocollagen thus preventing the assembly of fibrils. Fibrils, however, can be assembled if telopeptides are completely removed (Shoulders and Raines, 2009).

The first crystal structure of collagen-like peptide was reported in 1994 (Bella et al., 1994). The structure confirmed the presence of hydrogen interstrand bond between N-H group of glycine and C=O group of Xaa. The ladder of hydrogen bonds is important for holding the triple helical structure together. Its disruption leads to various pathological conditions. Triple helix is also stabilized through van der Waals interactions.

Repeating triplet of collagen dictates the staggering assembly of individual peptides within the helix. The second peptide is offset from the first by one amino acid, and the third – by two amino acids.

Glycine moiety is very important for the collagen properties. As a result of staggering, glycine residues are always located in the center of helix. They determine the unique peptide register which occurs in heterotrimeric helices. Heterotrimer consisting of two types of peptides can have three different register depending on which peptide is in leading, lagging and middle stands (AAB, BAA and ABA, correspondingly).

Mutations leading to glycine substitution are particularly disruptive in proline-poor sequences. This reflects the importance of proline moieties for the timely folding of collagen during the formation of triple helix. Formation of triple helix (nucleation) starts at proline-reach sites. Delays in folding result in overmodification of protocollagen such as hydroxylation of lysine residues and excessive glycosylation of the resulting hydroxylysine, leading to destabilization of triple helix. Introduction of just one alanine residue at fifth triplet in $(POG)_{10}$ sequence severely disrupt hydrogen bonding replacing it by water mediated contacts, decrease the melting temperature by 32^{o}C and leads to three-fold decrease of the folding rate of the triple helical assembly (Shoulders and Raines, 2009).

Prolyl-4-hydroxylase hydroxylates γ-carbon in the proline residues located at Yaa position of protocollagen. Hydroxylation dramatically increases the thermal stability of collagen triple helix. The degree of hydroxylation allows to modulate the thermal

stability of collagen. For example, mammalian collagen starts unfolding at around 37^{o}C while collagen from Antarctic ice fish denatures below 10^{o}C (Burjanadze, 2000).

Stability of triple helix structure depends on the nature of non-proline residues. Some amino acids, for example Arg at Yaa position, provide stability, while others such as aromatic amino acids have destabilizing effect (Yang et al., 1997).

Tropocollagen triple helices are capable of self-assembly into thicker (5 nm) and much longer collagen microfibrils. Microfibrils are the building blocks for very long (up to 1 cm) and thick (up to 500 nm) collagen fibers. Telopeptides play an essential role in further stabilization of mature microfibril structure via formation of crosslinks. Crosslinking provides additional strength and stability but is not needed for the initial formation of fibrils. Lysyl oxidase is playing an important part in this process of crosslinks formation. Crosslinks are formed both between different tropocollagen triple helices and between different fibrils, thus facilitating the formation of larger collagen fiber structures (Shoulders and Raines, 2009).

It seems that the length of collagen microfibrils (around 300 nm) is optimal for its mechanical properties. Both shorter and longer fibrils are less robust. Collagen fibers have better mechanical strength than microfibrils. Excessive crosslinking of telopeptides can lead, however, to brittle structures of collagen fibers that are becoming more common in the course of aging.

4. Function of collagens

Collagens play multiple important roles in normal functioning of the connective tissues. They provide mechanical strength of tissues and, at the same time, regulate its flexibility. Collagens are major building blocks of the scaffold for cell migration, growth and differentiation within the extracellular matrix.

Collagens also participate in interactions between cells and extracellular matrix which is mediated by specific receptors recognizing only triple helical structures of proteins (Vandenberg et al., 1991; Tuckwell et al., 1994). Remodeling of extracellular matrix requires collagen turnover. This is the process involved in a variety of normal and pathological processes and is mediated by matrix metalloproteinases. The latter enzymes require triple helical conformation of their substrates (Lauer-Fields et al., 2002).

Collagen has found multiple medical applications. It is used for developing of artificial skin, cartilage and bone. Excellent gel formation, biodegradation and cell scaffolding properties of collagen are instrumental for these applications.

5. Problems of using the natural collagen

Being one of the most abundant proteins in nature, collagen is easily available. Bovine collage, for instance, is commonly used in various biomedical applications. This is not, however, a good substitute for human protein: bovine collagen is heterogeneous since it loses its structural integrity in the course of isolation. This results in lower thermal stability and nonspecific cell attachment. Natural collagen has low solubility and is difficult to modify in a site-specific manner. Collagens of non-human origin can also

illicit pathological effects and cause the immune response activation when implanted in humans. Expression systems for human collagen do not solve the problem due to the need to introduce posttranslational modifications. Posttranslational modifications such as hydroxylation and crosslinking are of vital importance for formation of viable extracellular matrix.

Natural collagen is also difficult to study. Fiber diffraction does not reveal the structure of collagen fibrils with sufficient resolution. In addition, collagen molecules are too large, their polypeptide chains have repetitive structure, compound is insoluble and forms complex hierarchical structures. Modeling approach with the use of collagen-related peptides (CRP) is used from the 1960s to get insight into the structure of natural collagen (Fields and Prockop, 1996).

1.2 Overview of biomimetic collagen

The building blocks of natural collagen are long (around 1000 amino acids) polypeptides that undergo a variety of posttranslational modifications. The difficulties of obtaining and handling these polymers stimulated the attempts to find suitable low molecular weight substitutes.

Smaller synthetic peptides that adopt a triple helical structure can be used to study the molecular structure, stability and biochemistry of the collagen triple helix. Many of them demonstrate ability for further self-assembly into higher-order structures. For instance, concentrated aqueous solution of $(ProHypGly)_{10}$ consisting of repeats typical for natural collagen can assemble into branched fibril-like structures ((Shoulders and Raines, 2009).

The problem encountered in this field of research is the lack of understanding about the mechanisms and driving forces for hierarchical orderly formation of more complex microfibrils, fibers and hydrogel structures. Last two decades have seen multiple efforts in design and studies of various collagen mimicking peptides (CMPs) and their assembly into higher order nanostructures. Certain progress was achieved here and reliable methods of making these structures were developed. But many problems are still not addressed.

Most of research efforts focused on the studies of homotrimeric helical structures. Heterotrimeric structures gained some attention only in the last few years. Also, the successful formation of artificial CMP fibers and hydrogels still requires the use of peptides with various functional moieties and modifications. Biomimetic collagen-like assemblies still don’t have many essential characteristics of natural collagens. Synthetic collagens that are similar to natural collagen fibrils still have to be developed.

2. Design Synthesis and Characterization of collagen mimetic peptides

2.1 Homotrimeric synthetic collagen

The methods for preparation of high stability synthetic collagen developed in the last decades utilize a multitude of approaches. The formation of triple helices can be driven by amino acid propensity (Shah et al., 1996; Persikov et al., 2000; Persikov et al., 2005), electrostatics (Persikov et al., 2005; Gauba and Hartgenik, 2007a; Gauba and

Hartgenik, 2007b; Gauba and Hartgenik, 2008; Venugopal et al., 1994; Fallas et al., 2009), hydrophobicity (Shah et al., 1996; Kar et al., 2009), cysteine knots (Krishna and Kiik, 2009;) and natural collagen sequences (Madhan et al., 2008). Some of the most important and productive approaches will be discussed below.

2.1.1 Synthesis

Although collagen related peptides tend to self-assemble into larger structures, the triple helices derived from them are usually much shorter (approximately 10 nm) than natural tropocollagen (300 nm). The problem of insufficient length was first addressed by Kotch and Raines (Kotch and Raines, 2006) by introducing the self-assembly system based on the use of covalently conjugated “sticky end” peptides. The peptides were contained cysteine residues that allow formation of inter-peptide covalent disulfide links holding three peptides together. This “sticky end” fragments can assemble via intermolecular formation of triple helix yielding the fibrils that are even longer than natural collagen (400 nm and above). Disulfide bonds of this so called cystine knot not only keep peptides together but also set a register for self-assembly. It can be modified by changing the positions of cysteine moieties.

A variety of other approaches yielding long triple helices imitating natural collagen were introduced since then.

One of the approaches is based on π-stacking interaction between electron-rich phenyl group of C-terminal phenylalanine residue with electron-poor pentafluorophenyl group of N-terminal pentafluorophenylalanine residue of CMP (Cejas, et al., 2007). The approach produces micrometer-long structures that were used for make thrombogenic collagen-imitating fibrils.

Kishimoto and co-workers (Kishimoto et al., 2005) suggested a simple chemical process for preparation of long fibrous collagen. The process is based on polycondensation of $(ProHypGly)_n$ oligopeptides (n = 1, 5, 10) in the precence of chemical agents facilitating the formation of amide bond between peptides. The approach allows to synthesize collagen polypeptides with molecular weights above 10,000 Da. The polypeptides obtained this way were thermally stable up to the temperature of 80^oC. Moreover, they were able to aggregate with formation of larger nanofiber-like structures about 10 nm in width.

2.1.2 Characterisation

A variety of physicochemical methods can be utilized to characterize synthetic analogues of collagen and self-assembled structures produced in the course of experiments. Examples of these techniques include CD spectroscopy, dynamic light scattering, analytical ultracentrifugation, transmission electron microscopy and atomic force microscopy. The atomic resolution details can be obtained with the use of X-ray analysis and modern NMR methods, although the application of these methods is limited to smaller synthetic structures.

Folding of collagen mimicking peptides is usually studied by circular dichroism (CD) spectroscopy. CD spectra of triple helices show a typical maximum near 225 nm and a minimum in the region of 190 nm. Circular Dichroism spectroscopy allows to visualize the denaturation process of proteins and measure the unfolding temperature (melting temperature) of triple helices formed during the self-assembly of synthetic collagen related peptides. Melting temperature is a major measure of stability. It is obtained by studying the temperature dependence of CD spectra and generating a melting curve.

Dynamic light scattering (DLS) helps in estimating the size of macromolecules or their assemblies. DLS can measure the diffusion coefficient of macromolecules in the solution. Diffusion coefficient provides information about the molecular weight size and shape of macromolecule. Distribution of diffusion coefficient gives some ideas about the range of macromolecule sizes in the mixture (the size is estimated via Stokes radius). Measuring the changes of diffusion coefficient, it is possible to obtain information about the dynamics of the system and speed of changes. Analytical ultracentrifugation can provide similar information. Comparison of results obtained by two methods helps to see if they correlate and therefore reliable.

Analytical ultracentrifugation can give information about the number of complexes or components existing in the solution. It also provides information about reversibility of their interaction. Since asymmetric complexes tend to sediment slower, information about the molecular asymmetry can be obtained through sedimentation coefficients. Sedimentation coefficients also point out on the presence and number of multimeric assemblies (larger assemblies always have larger sedimentation coefficients).

The morphology of macromolecular assemblies can be studies be atomic force microscopy (AFM) and transmission electron microscopy (TEM). Long linear fibrils of triple helical collagens and their synthetic analogues can be easily seen and measured by these methods.

2.2 Heterotrimeric synthetic collagen

Most research in regards to synthesis and assembly of collagen mimetics were done on homotrimeric models. However, heterotrimeric triple helices are more common in nature than homotrimeric collagens. This indicates the existence of certain advantages of heterotrimeric configuration, possibly related to higher functional diversity and possibility to bind specific biomolecules, enzymes and receptors.

In comparison with homotrimeric assemblies, design of heterotrimeric helices faces two problems: control of composition and register. Simple mixing of two or three different collagen-like peptides is unlikely to produce a single heterotrimeric product. Peptides A and B can be associated as either A_2B or AB_2 complexes, in addition to formation of homotrimers A_3 and B_3. Due to one register stagger observed in natural collagen, the heterotrimer AAB can form 6 possible structures, and heterotrimer ABC – up to 27 different triple helices. The process of selective formation of a particular type of heterotrimeric helices is poorly understood. The synthetic approaches leading to preferred formation of a single collagen-like heterotrimeric configuration rely on formation of structures with the highest energetic stability.

2.2.1 Covalently linked heterotrimers

The majority of approaches for preparation of covalently linked heterotrimers is utilizing the regioselective incorporation of cysteine residues into CMP with subsequent formation of disulfide bridge (Ottl et al., 1998). Synthetically and technically, the approach is similar to the same cysteine knot method described earlier for formation of homotrimeric helices.

Li et al. (2011) reported AAB triple helix forming system that uses a technique to covalently link the peptides together thus driving formation of heterotrimer.

2.2.2 Self-assembled heterotrimers

In 2010, Russell and co-workers reported the first selective self-assembly of heterotrimeric AAB collagen (Russell et al., 2010). The researchers mixed together, in 2:1 ratio, two peptides that have canonical $(XaaYaaGly)_n$ composition but differ in charges. More abundant peptide (A: $(EOGPOG)_5$) has half of the charge opposite to the change of second peptide (B: $(PRG)_{10}$). This results in the formation of AAB heterotrimeric complex. Formation of other possible helices (AAA, ABB and BBB) is energetically unfavourable due to electrostatic repulsion. On the contrary, formation of AAB complex leads to the stabilisation of structure. The authors have shown that heterotrimer formed this way has higher thermal stability (melting temperature is 10^{o}C higher compared to homotrimeric AAA triple helix).

Artificial preparation of ABC type of heterotrimeric collagen represents even more challenging task. In 2007, the preparation of stable heterotrimer ABC was described (Gauba and Hartgenik, 2007a; Gauba and Hartgenik, 2007b). Heterotrimer consisted on negatively charged $(DOG)_{10}$ in the middle chain, positively charged $(PKG)_{10}$ in leading chain and neutral peptides $(POG)_{10}$ in lagging chain. The major drawback on this approach was the formation of highly stable homotrimer consisting of neutral $(POG)_{10}$ peptides thus reducing the homogeneity of heterotrimer solution.

Fallas and co-workers (Fallas et al., 2011) reported the rational design approach to the synthesis of single-composition ABC heterotrimer. The approach is an extenuation of the earlier work from this group (Russell et al., 2010) described in the previous paragraph. Individual peptides for self-assembly were designed in such a way that one of the peptides carries a positively charged amino acid every third position plus a single negatively charged amino acid at certain position of each repeat, another peptide has negatively charged residues at every third position, and the third one is neutral except one position in the repeat which is positively charged. Placing charged amino acids at certain position allows to set a register specifically. Interaction between negatively and positively charged strands lowers down the energy of heterotrimer formed.

Initial experiments described in this paper were using a completely neutral third peptide but again as in previous experiments the homotrimer consisting of these neutral peptides was a preferred conformation. To address this problem, researchers have changed the sequence of amino acids for the uncharged peptide introducing a single charged moiety. This modification results in the formation of homotrimer with lower thermal stability. This so-called negative design approach helps to widen the energetic gap between different configurations and shift the equilibrium towards the

desirable heterotrimer. Reducing the number of stabilizing POG repeats, in addition to introducing the charge repulsion, has also improved the specificity of heterotrimer formation (Lesley et al., 2011).

A computational approach to the design of collagen mimetics capable of self-assembly was recently reported (Fallas and Hartgerink, 2012). The method allows to predict successfully the best possibly sequences capable of register-specific heterotrimer formation.

2.3 Hierachical self-assembly of collagen into nanofibres and hydrogels using CMPs

2.3.1 Synthesis

In general, self-assembling peptide hydrogels are easy and straightforward to prepare. These hydrogels are becoming a popular choice of material for various biomedical applications such as drug delivery, cell delivery and scaffold for tissue regeneration and engineering (Bacota et al., 2011). They can form nanostructures self-assembling into hydrogels with a variety of properties. Properties can be modified by the choice of peptides chemical composition and by further modifications such as enzymatic crosslinking. The latter improves rheological characteristics of the hydrogels.

Non-collagenous peptide-amphiphiles were demonstrated to assemble into nanofibers with formation of hydrogels under a variety of conditions (Hartgerink et al., 2002). Although this system does not produce collagen fibers, it allowed to study the general mode and mechanisms of self-assembly. The system is important in understanding the factors influencing more biologically relevant collagen mimicking peptides.

In contrast with simple peptide based hydrogel, preparation of hydrogels based on collagen mimetics turned out to be much more difficult task. Without some kind of chemical modifications, the solutions of these peptides usually do not show gelation, even when nanostructures formation is observed. Modification of triple helix building blocks, however, helps to achieve the formation of hydrogels Yamazaki et al. (2008) reported the self-assembly of artificial “sticky end” peptides linked by disulfide bonds up to the level of hydrogel.

Native collagen forms quasi-hexagonal lattice consisting of five triple helices and capable of further self-assembly into various forms of mature collagen fibers. As a result of staggering packaging, a characteristic banding pattern (D-banding) is observed in collagen fibers. Typical length of D-bands is 64-67 nm. At present time, none of the artificial systems can fully imitate this unique organization of natural collagen.

Most of the collagen mimicking triple helical structures do eventually form agglomerates and precipitate from the solutions. Their structure can be studied by microscopic methods such as atomic force microscopy, scanning electron microscopy and transmission electron microscopy. In most cases some disorganized mesh-like assemblies can be seen with the use of these methods. This indicates the absence of the driving force that would organize triple helices into more orderly higher level structures (Fallas et al., 2010).

There were several approaches published in the literature that aimed to drive the organized fiber formation. Nanoscale sized fibers can be assembled with the use of modified peptides flanked by hydrophobic residues, such as tyrosine and phenylalanine, at C- and N-termini (Cejas et al., 2007; Kar et al., 2009). π-Interaction between aromatic amino acid residues and proline or hydroxyproline is thought to be a driving force of this fiber formation.

The use of cystine knots is another approach facilitating the formation of nanostructures. Cysteine residues are placed insude the long collagen mimicking peptide sequences. Once oxidized, they form covalent bonds to each other thus facilitating crosslinks formation. In the systems containing cysteine the formation of hydrogel was observed but microscopic investigation didn't reveal the presence of organized structures (Yamazaki et al., 2008). Another cysteine knot approach described earlier (two cysteine residues are located at the C-termini of two peptides while the third peptide contains two cysteine moieties at various internal position) creates "sticky end" proteins capable of forming triple helices and more complex nanostructures (Kotch and Raines, 2006). Alternative chemical approach involves incorporation of cysteine at N-terminus and thioester at C-terminus of peptides. In this case, chemical ligation rather than disulfide bond formation drive head to tail polymerization which produces highly organized nanofibers (Paramonov et al., 2005).

The earlier mentioned approach based on π-stacking interaction between electron-rich phenyl group of C-terminal phenylalanine residue with electron-poor pentafluorophenyl group of N-terminal pentafluorophenylalanine residue produces micrometer-long fibrils (Cejas, et al., 2007). These fibrils were used for make thrombogenic collagen-imitating fibrils. Electricity-conducting nanowire were made on the basis of these fibrils by coating them with gold nanoparticles and plating with silver (Gottlieb et al., 2008).

Head to tail polymerization-based self-assembly can be mediated by divalent metals, as reported by Pires and Chmielewski (Pires and Chmielewski, 2009). The resulting higher order structures, however, are not fibers but microflorettes instead.

Hydroxyproline-lacking collagen mimicking peptides are not particularly keen on forming the triple helices. One of the recent reports, however, shows that some sequences carrying cysteine residue at C-termini and having the possibility of electrostatic interaction within homotrimer do form triple helices and nanofibers (Krishna and Kiik, 2009)).

Incorporation of electrostatic interaction within the trimer can also be a driving force for the formation of fibers. Chaikof and Conticello group (Rele et al., 2007) used peptide with the sequence $(PRG)_4(POG)_4(EOG)_4$ containing positively charged arginine residue in the N-terminal region and negatively charged glutamate residue in the C-terminal region to form highly organized 3-4 μm long nanofibers. Upon thermal treatment the researchers observed the formation of thicker fibrils (70 nm in diameter compared to original 12-15 nm). The process of self-assembly in this case relies on electrostatic interaction between cationic and anionic blocks (arginine and glutamate residues). An impressive feature of these fibers was the presence of D-banding. D-period was 18 nm, which is significantly smaller than 64-67 nm of native collagen, but the authors explain this by the significant difference in the lengths of building blocks in

these two cases. This is the only collagen mimicking peptide-based nanostructure with D-periodicity reported so far.

Many of the reported collagen mimicking peptide systems that are capable to form nanostructures do not progress further to the gelation step, while some others show gelation in the absence of nanofibers or triple helices. The nanofibers reported by Rele and co-workers (Rele et al., 2007) require specific conditions for preparation and do not form hydrogels. O'Leary and co-workers (O'Leary et al., 2011) modified the collagen mimicking peptide used in the work of Rele et al. by replacing arginine with lysine and glutamate with aspartate. The resulting sequence $(PKG)_4(POG)_4(DOG)_4$ still features the same characteristics since it has zwitterionic structure and contains charged amino acids at the same positions. The rationale behind these changes was to introduce a modified network of hydrogen bond interactions and salt bridges within the triple helix. The design was based on the results of NMR studies of several heterotrimeric collagen complexes. The changes brought more effective interaction between lysine and aspartate which lead to the superior nanofiber which were able to interact with formation of hydrogels. AFM, SEM and TEM (both dry and hydrated techniques) all confirmed the presence of nanofibers. Hydrogels self-assembles from these nanofibers have shown good viscoelastic properties comparable to natural hydrogels. The obtained hydrogel was successfully broken down by collagenase type IV (matrix metalloproteinase) at the rate similar to the digestion of natural collagen. The results point out on rather close similarity of this collagen mimicking peptide self-assembly to the process observed for natural collagen (O'Leary et al., 2011).

Currently there is the only system available based on small collagen mimicking peptides that can self-assemble into nanofibres and form hydrogels. Such system could be used as the mimic of extracellular matrix and have multiple biomedical application.

None of the heterotrimeric helical systems designed so far can assemble further beyond triple helix and form any nanofibers. More research needs to be done to address this issue.

Although most of the studied collagen mimetic peptides do not form hydrogels, they can be successfully used for tissue engineering. An interesting approach which helps to increase the tissue production by chondrocytes was developed by Lee and co-workers (Lee et al., 2006). The researchers conjugated poly(ethyleneglycol) (PEG) based hydrogel with photopolymerizable collagen mimicking peptides. They found that when chondrocytes were encapsulated in this hydrogel and incubated for extended period of time, the collagen production increased significantly compared to unmodified PEG hydrogel. The authors assume that collagen mimicking peptides provide collagen binding sites which simulate natural extracellular matrix. The peptides are likely to invade natural collagen by strand displacement thus providing the sites for further nanofibers assembly and physical crosslinks that increase the strength of ECM. This hypothesis is confirmed by findings of Mo et al. (2006) who demonstrated that collagen mimicking peptides bind preferentially to the gap regions on the surface of intact natural collagen I fibers.

2.3.2 Characterisation

TEM and AFM show that in all cases where formation of fibers was observed, the fibers represented only a minority of structures in the samples. The rest of the system looks like amorphous aggregates. TEM and AFM use dry techniques. In the course of sample drying, the samples experience a significantly changing condition, in particularly the increase in peptide and salt concentration. This may have an impact on the formation of nanostructures. Wet techniques such as ice cryo-TEM have never confirmed the presence of fibers in the cases discussed earlier. Vitreous ice cryo-TEM is much more reliable method since it shows what is going on in the system without any effect caused by drying. The majority of fibers reported for collagen related peptides show high amount of amorphous aggregation on the TEM images and low uniformity of assembled materials. Clearly, optimal conditions for formation of fibrils are not yet known (Fallas et al., 2010).

3. Conclusion

3.1 Existing fields

Significant progress in our understanding of collagen structural organization has been achieved in the resent years. This advance in knowledge was helped, in large degree, by research focused of the self-assembly of collagen mimetic peptides. Now we have much better understanding of the complex mechanics behind the hierarchical formation of microfibrils, collagen fibers and hydrogels. The research also opened the possibility of preparing various materials that can be used for practical biomedical applications and tissue engineering.

3.2 Future areas of research

Successful applications of natural collagens and their mimetics in biomedical and biomaterial fields require better methods for their preparation.

Better understanding of structure and stability of heterotrimeric collagen is needed. First examples of heterotrimeric structures capable to self-assembly to the level of hydrogels were reported, but much more has to be done to achieve flexibility and predictability of these approaches. The ability to easily produce high quality fibrils and control their formation still remains to be achieved. Heterotrimeric nanofibers capable of hydrogel formation have not yet been produced.

More knowledge needs to be gained in a very important area of interaction between collagen and other biomolecules and developing the systems that facilitate and promote this interaction.

Key step for biomedical applications of CMPs is the development of mimetics capable of binding to natural collagen. Some encouraging results have been published recently, but they still remain isolated examples of what can be done here.

Most research in recent years was focused on fibrillar collagen and its minetics. The structure and formation mechanism of nonfibrillar collagen structures remain poorly understood. Further structural and mechanistic studies are needed in this area.

References:

Bakota, E.L. et al. (2011) Enzymatic cross-linking of a nanofibrous peptide hydrogel. Biomacromolecules 12, 82-87.

Bella, J. et al. (1994) Crystal and molecular structure of a collagen-like peptide at 1.9 A resolution. Science 266, 75-81.

Burjanadze, T.V. (2000) Biolopymers 53, 523-528.

Cejas, M. et al. (2007) Collagen related peptides: self-assembly of short, single strands into functional biomaterials of micrometer scale. J. Am. Chem. Soc. 129, 2202-2203.

Fallas, J.A. et al. (2009) J. Biol. Chem. 284, 26851-26859.

Fallas, J.A. et al. (2010) Synthetic Collagen Mimics: self-assembly of homotrimers, heterotrimers, and higher order structures. Chem. Soc. Rev. 39, 3510-3527.

Fallas, J.A. and Hartgerink, J.D. (2012) Computational design of self-assenbling register-specific collagen heterotrimers. Nature Communications, 3: 1087

Fallas, J.A. et al. (2012) Rational design of single composition ABC collagen heterotrimers. J. Am. Chem. Soc. 134, 1430-1433.

Fields, G.B. and Prockop, D.J. (1996) Perspectives on the synthesis and applications of triple-helical collagen-model peptides. Biopolymers 40, 345-357.

Galler, K.M. et al. (2012) A customized self-assembling peptide hydrogel for dental pulp tissue engineering. *Tissue Engineering: Part A* 18, 176-184.

Gauba, V. and Hartgenik, J.D. (2007a) J. Am. Chem. Soc. 129, 2683-2690.

Gauba, V. and Hartgenik, J.D. (2007b) J. Am. Chem. Soc. 129, 15034-15041.

Gauba, V. and Hartgenik, J.D. (2008) J. Am. Chem. Soc. 130, 7509-7515.

Gottlieb, D.G. et al. (2008) Self-assembled collagen-like peptide fibers as templates for metallic nanowires. J.Mater. Sci. 18, 3865-3870.

Hartgerink, J.D. et al. (2002) Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. Proc. Natl. Acad. Sci. USA 99, 5133-38.

Kar, K. et al. (2009) Biochemistry 48, 7959-7968.

Kishimoto, T. et al. (2005) Synthesis of Poly(Pro-Hyp-Gly)$_n$ by direct polycondensation of (Pro-Hyp-Gly)$_n$, where n = 1, 5 and 10, and stability of the triple helix structure. Biolopymers 79, 163-172.

Krishna, O.D. and Kiik, K.L. (2009) Biomacromolecules 10, 2626-2631.

Koide, T. (2007) Designed triple-helical peptides as tools for collagen biochemistry and matrix engineering. Phil. Trans. R. Soc. B 362, 1281-1291.

Kotch, F.W. and Raines, R.T. (2006) Self-assembly of synthetic collagen triple helices. Proc. Natl. Acad. Sci. USA 103, 3028-3033.

Lauer-Fields, J.L. et al. (2002) Biopolymers 66, 19-

Lee, H.J. et al. (2006) Collagen mimetic peptide-conjugated photopolymerizable PEG hydrogel. Biomaterials 27, 5268-5276.

Li, Y. et al. (2011) Biopolymers 95, 94-104.

Madhan, B. et al. (2008) J. Am. Chem. Soc. 130, 13520-13521.

Mo, X. et al. (2006) Nanoparticle-assisted visualization of binding interactions between collagen mimetic peptide and collagen fiber. Angew. Chemie Int. Ed. 45, 2267-2270.

O'Leary, L.E.R. et al. (2010) Positive and negative design leads to compositional control in AAB collagen heterotrimers. J. Am. Chem. Soc. 133, 5432-5443.

O'Leary, L.E.R. et al. (2011) Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel. Nature Chemistry 3, 821-828.

Ottl, J. et al. (1998) FEBS Letters 398, 31

Paramonov, S.E. et al. (2005) Macromolecules 38, 7555-

Persikov, A.V. et al. (2000) Biochemistry 39, 14960-14967.

Persikov, A.V. et al. (2005) Biochemistry 44, 1414-1422.

Pires, M.M. and Chmielewski, J. (2009) J. Am. Chem. Soc. 131, 2706-

Rele, S. et al. (2007) J. Am. Chem. Soc. 129, 14780-

Russell, L.E. et al. (2010) Selective Assembly of a high stability AAB collagen heterotrimer. J. Am. Chem. Soc. 132, 3242-3243.

Shah, N.K. et al. (1996) Biochemistry 35, 10262-10268.

Shoulders, M.D. and Raines, R.T. (2009) Collagen structure and stability. Annu Rev Biochem 78, 929-958.

Tuckwell, D.S. et al., (1994) J. Cell. Sci. 107, 993-

Vandenberg, P. et al. (1991) J. Cell. Biol. 113, 4175-

Venugopal, M.D. et al. (1994) Biochemistry 33, 7948-7956.

Yamazaki, C. et al. (2008) Artificial collagen gels via self-assembly of de novo designed peptides. Biopolymers: Peptide Science 90, 816-.

Yang, W. et al. (1997) Gly-Pro-Arg confers stability similar to Gly-Pro-Hyp in the collagen triple-helix of host-guest peptides. J. Biol. Chem. 272, 28837-28840.