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Multicomponent gels: modeling structure with low-molecular-weight gelators

SUMMARY OF DOCTORAL THESIS

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With special consideration,

Alexandra Croitoriu

CONTENTS

INTRODUCTION

Supramolecular chemistry is the discipline that deals with the study of intermolecular bonds [1], being also known as "chemistry beyond molecules" [2]. This field is based on the study of molecular recognition and highly ordered self-assembly generated by secondary or reversible covalent molecular interactions [3]. In 1987, supramolecular chemistry was recognized and accepted as an important branch of chemistry thanks to the studies undertaken by the group formed by Charles J. Pedersen, Jean-Marie Lehn, and Donald J. Cram, a group that received the Nobel Prize in Chemistry for "the development and the use of molecules with specific and highly selective structural interactions" [4]. An interdisciplinary field, supramolecular chemistry refers to the formation of supramolecular structures through the aggregation of molecular chains according to complementary functional groups, their conformation and configuration, as well as through the influence of external factors [5] [6].

Nature and biological systems are dominated by molecular self-assembly processes through which complex supramolecular structures with distinct functionalities are formed. Deoxyribonucleic acid (DNA), proteins, extracellular matrix (ECM), and even viruses are created by molecular recognition and highly ordered self-assembly of amino acids, sugars, metal ions, and nucleic acids [7]. Widespread in nature, secondary molecular interactions are of particular importance in the hierarchical organization of biological systems and in supporting their functionality by providing flexibility and specificity in biological processes [8] [9]. Although secondary molecular interactions have a weak bond energy compared to covalent bonds, they can form stable structures because of the synergistic effect of intra- and intermolecular interactions [9].

Although low-molecular-weight gelators (LMWGs) were discovered in the early 19th century, the supramolecular nature of these materials was poorly understood and remained unstudied until the late 20th century. Molecules with a great structural diversity, from the simplest alkanes to complex peptides, have been shown to be LMWGs. The **motivation** for the research direction addressed in the doctoral thesis is based on an understanding of how molecular aggregates are formed at different levels and exploring their potential for innovative technological applications.

Starting from these considerations, the **general objective of the doctoral thesis** entitled **"Multicomponent gels: modeling structures with low-molecular-weight gelators"** consisted in the design, obtaining and optimization of new polymer systems based on LMWGs and natural and/or synthetic polymers, with stable structure, physico-chemical properties and applicative potential as a support matrix for the growth and development of three-dimensional cell cultures.

The thesis is structured in two parts and includes seven chapters with appendices and references.

Part I of the doctoral thesis includes an introductory chapter, *Chapter I,* dedicated to the literature study regarding the analysis of the behavior of amino acids to self-assemble/co-assemble under specific conditions as well as the driving forces involved in the generation of supramolecular structures, as well as the current state of research in the field of gels based on LMWGs and natural/synthetic polymers with applicability in the medical field.

Part II of the thesis is structured in six chapters and presents the original contributions regarding the obtaining process, physicochemical characterization and *in vitro/in vivo* testing of the obtained supramolecular systems.

Chapter II includes the study of amino acids and short peptides ability to form supramolecular structures, being divided into two subchapters describing: (i) the design and characterization of supramolecular systems by self/co-assembly of some amino acids and short peptides under the influence of pH change and (ii) the design and characterization of supramolecular systems by self/co-assembly of amino acid pairs following the use of a polar aprotic solvent. Also, this chapter highlights the importance of the fluorenylmethoxycarbonyl (Fmoc) group found at the N-terminus of the compounds used, as well as the additional driving forces that support gelation and the formation of three-dimensional structures. The obtained supramolecular systems were structurally characterized both in solution and in the solid state, highlighting the association dynamics and the assembly mechanism underlying the structuring process. The morphological peculiarities were highlighted by microscopy techniques, while the characterization of the systems in the gel state allowed the viscoelastic behavior to be identified. In order to establish the potential application as a support matrix for the growth and development of three-dimensional cell cultures, the prepared supramolecular systems were tested *in vitro* and *in vivo* from a cytotoxic point of view.

Chapter III includes the preparation and physico-chemical characterization of multicomponent hydrogels that were obtained by integrating natural gelling polymers (gellan gum, respectively agarose) in the co-assembled supramolecular matrices synthesized in Chapter II. By adding the natural components, the aim was to maintain the biocompatible character, but also to improve the supramolecular network by increasing the consistency and strength of the matrices obtained. This chapter presents the preparation and characterization of two types of multicomponent systems: (i) multicomponent gels based on supramolecular structures and gellan gum and (ii) multicomponent gels based on supramolecular structures and agarose. Both variants of the multicomponent gel were characterized structurally, morphologically, rheologically and cytotoxically.

Chapter IV presents the preparation and characterization of new hybrid hydrogels of semi-interpenetrated type (semi-IPN) containing supramolecular gels together with natural and synthetic polymers. In this sense, we made the synthetic copolymer poly(itaconic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5]undecane) – (PItAU), step followed by obtaining the bioconjugate based on sodium alginate (NaAlg) and PItAU by involving the hydroxyl functional groups of sodium alginate and opening the cycle of itaconic anhydride and finally, the preparation of hybrid gels by the interaction between the bioconjugate and the supramolecular system. The formation of the copolymer was confirmed by the performed spectral analyzes (^{1}H-NMR, FTIR), and the formation of the bioconjugate was validated by FTIR spectroscopy. The hybrid gels were subjected to spectral, morphological and rheological analyses, but also to biological analyzes performed *in vitro* and *in vivo*.

Chapter V describes the "*in situ*" preparation and characterization of double network (DN) gels based on poly(2-(dimethylamino) ethyl methacrylate) and supramolecular structures. Obtaining the double-network gels was carried out in two steps: (i) initially the poly(2-dimethylaminoethyl methacrylate) polymer matrix was formed by radical polymerization in the presence of the ammonium persulfate/N-N-N'-N'- tetramethyl-ethylenediamine (TEMED) redox initiation system and cross-linking of the homopolymer with N-N'-methylene-bis-acrylamide in water and (ii) interpenetration with a second supramolecular network formed by co-assembling an amino acid with a short peptide. The DN network of the gels was confirmed by corroborating the results obtained from the spectral, morphological and rheological analyses.

Chapter VI contains general information about the materials used in the experimental study to obtain each type of gel, but also the equipment used to characterize the compounds obtained.

The last chapter of the thesis, *Chapter VII,* includes the general conclusions regarding the obtained experimental results, as well as perspectives in the field of supramolecular gels based on LMWGs and natural/synthetic polymers.

The doctoral thesis entitled **"Multicomponent gels: modeling structures with low-molecular-weight gelators"** extends over 237 pages and is structured in 7 chapters that include 45 tables, 117 figures and 255 bibliographic references, and at the end the following appendices are found:

Annex 1. Dissemination of results and scientific activity carried out within research projects;

Annex 2. Bibliographic references;

Annex 3. Scientific articles.

PART II – EXPERIMENTAL STUDY

CHAPTER II
SUPRAMOLECULAR GELS BASED ON AMINO ACIDS AND PEPTIDES AS LOW-MOLECULAR-WEIGHT GELATORS

2.1. The context of the developed research

Supramolecular gels based on amino acids/peptides are materials with particular importance in biomedical applications due to their outstanding intrinsic properties. The design of supramolecular systems is based on the formation of highly ordered structures as a result of the hierarchical self-assembly process carried out through non-covalent interactions. The main advantages presented by these materials are related to the biocompatible, bioreactive and bioadhesive nature of amino acids, supporting cell proliferation. These characteristics, together with the nanofibrous appearance and high hydration capacity, are essential for the development of three-dimensional structures with a role in cell growth.

By introducing a co-partner (amino acid or peptide) it is proposed to improve the rheological, morphological and structural properties of the hydrogels resulting from the interaction between the two different components. In addition, the identification of the driving forces involved in the generation of three-dimensional supramolecular structures of amino acids is another aspect that requires special attention.

2.2. Objectives of the experimental study

In this context, the study regarding the supramolecular gels obtaining focused on the design of a supramolecular amino acid-peptide or amino acid-amino acid system with stable structure, physical and chemical properties and applicability in the development of cell cultures. The present study aimed at: *obtaining self-assembled/co-assembled supramolecular gels based on amino acids and N-terminally functionalized peptides with the aromatic Fmoc group*. The necessary conditions for the fulfillment of the targeted objectives are:

- Ability determination of amino acids and short peptides to act as LMWGs;
- Studies on the analysis of the behavior of amino acids to self-assemble under specific conditions (pH, solvent);
- Supramolecular gels obtaining by co-assembling different pairs of functionalized amino acids or by combining amino acids with short-chain peptides;
- Determination of the application potential of supramolecular systems in the biomedical field.

2.4. The design of supramolecular gels

Following the steps of ability determination of amino acids and short peptides to self/co-assemble, four stable systems were chosen to be further evaluated and characterized. The selected systems are shown in table 2.3.

Obtaining supramolecular gels was based on triggering the gelation process by two methods: changing the pH (*subchapter 2.4.1*) and by using a polar aprotic solvent (*subchapter 2.4.4*).

Tabel 2.3. Geluri co-asamblate selectate pentru evaluare și caracterizare.

CO-ASSEMBLED SYSTEM			
System code	**Minimum gelling concentration**		**Variant**
	Amino acid	**Co-partener**	
S_1 : Fmoc-Trp-OH_ Fmoc-Lys(Fmoc)-OH	0.5%	0.5%	V_4 +V_2*
S_2 : Fmoc-Lys(Fmoc)-OH_Fmoc-Ser-OH	0.5%	0.1%	V_2 + V_1*
S_3 : Fmoc-Lys(Fmoc)-OH_Fmoc-Glu	0.5%	0.1%	V_2 + V_1*
S_4 : Fmoc-Lys(Fmoc)-OH_Fmoc-Gly-Gly-Gly-OH	0.5%	0.1%	V_2 + V_1*

2.4.1. Obtaining supramolecular gels based on tryptophan and lysine by pH modification

The supramolecular gel based on **Fmoc-Trp-OH** (M_1) and **Fmoc-Lys(Fmoc)-OH** (M_2) was obtained by changing the pH and will be found hereafter under the name **S_1 supramolecular system** followed by the ratio between the two co-partners (1:3, 1:1 and 3:1). After 24 hours of preparation the samples had a transparent appearance.

To determine the molecular arrangement, the supramolecular gels were structurally characterized in solution by DLS, CD, FL, UV-VIS, but also in the gel state by rheology studies. Furthermore, the systems were lyophilized, thus allowing structural (FTIR, XRD) and morphological (SEM, POM, AFM, STEM) characterization. The bioapplicative potential was determined by *in vitro* and *in vivo* tests.

The performed analyzes demonstrated the structuring and co-assembly of the two co-partners in a three-dimensional morphology, by alternating the ratio of Fmoc-Trp-OH and Fmoc-Lys(Fmoc)-OH. The molecular arrangement was facilitated by *π-π interactions* and stabilized by *hydrogen bonds* between –CO–NH– groups. Moreover, the X-ray diffraction revealed that the XRD diffractogram of the M1 self-assembled gel shows a characteristic aspect of isotropic structures, and by co-assembling in a 1:1 ratio with Fmoc-Lys(Fmoc)-OH, a more ordered molecular organization occurs.

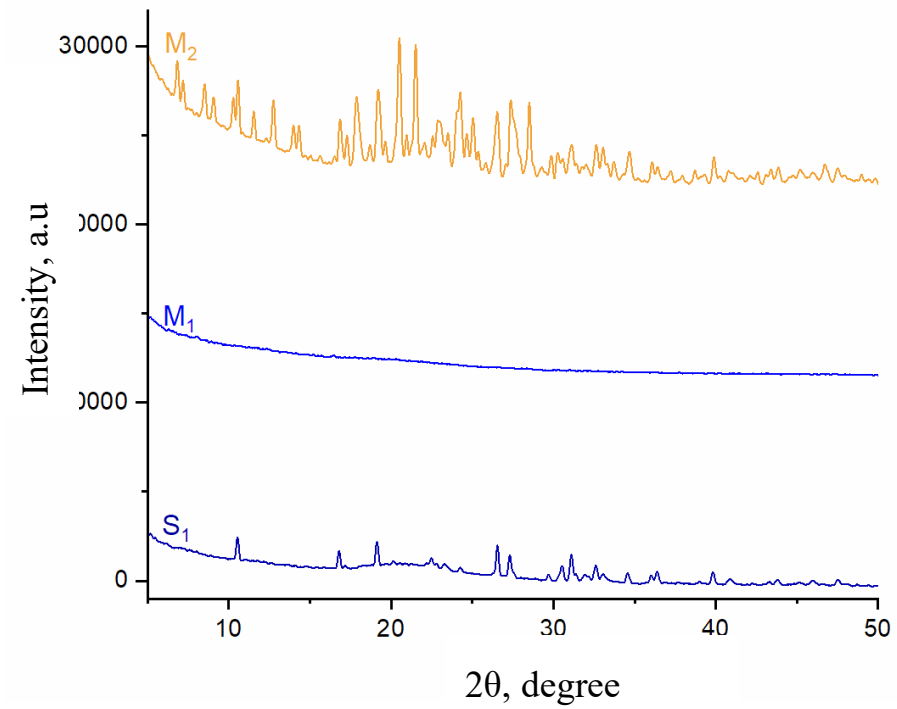


Figure 2.7. XRD diffractograms of the precursor compounds (Fmoc-Trp-OH and Fmoc-Lys(Fmoc)-OH) and the S_1 co-assembled system in a 1:1 ratio.

The complex morphological study shows that the 1:1 co-assembled S_1 system and its precursors exhibit fibrillar morphology generated by ordering processes, which continue with time and in dry films.

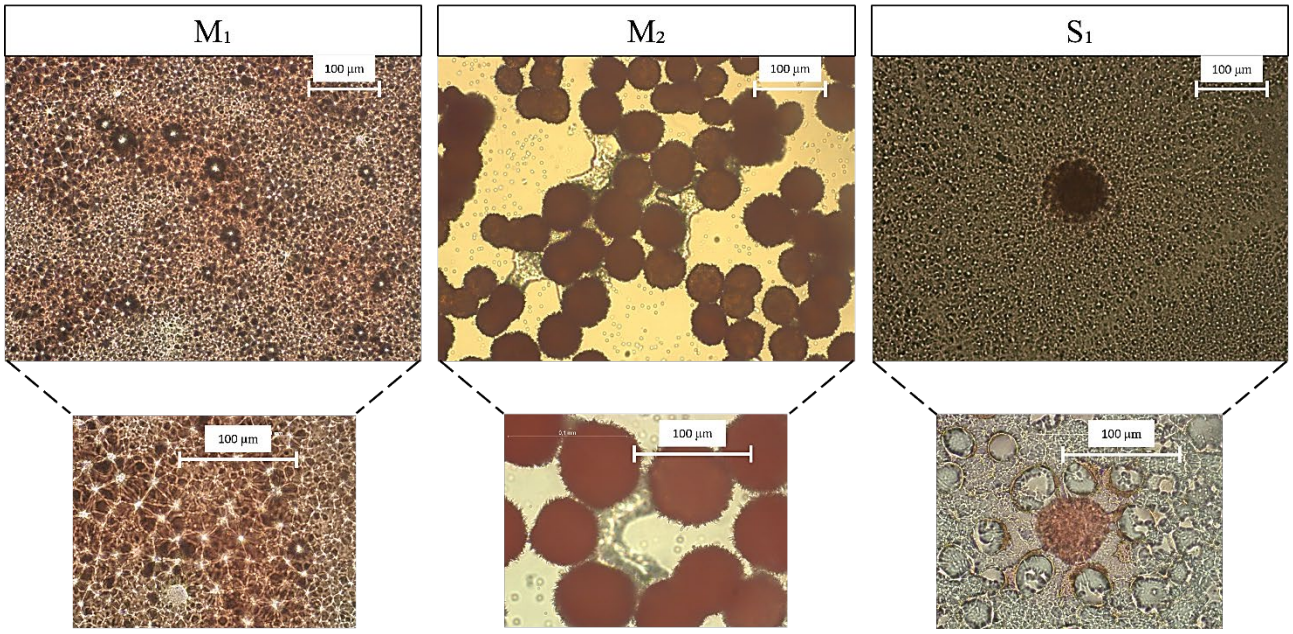


Figure 2.12. POM images of S_1 co-assembled system and Fmoc-Trp-OH (M_1) and Fmoc-Lys(Fmoc)-OH (M_2) precursors. Scale bar represents 100 µm.

From the rheological studies it was observed that the co-assembled systems, with the exception of the 3:1 ratio, exhibit behavior characteristic of gels with $G' > G''$ and $\tan \delta < 1$. This behavior is observed in samples with a high content of Fmoc-Lys (Fmoc)-OH: S_1 at a ratio of 1:3 and 1:1. Also, the viscoelastic modulus decreases as the Fmoc–Trp-OH content increases. This behavior can be

correlated with the presence of indole rings that create space between the molecules and diminish their potential to interact.

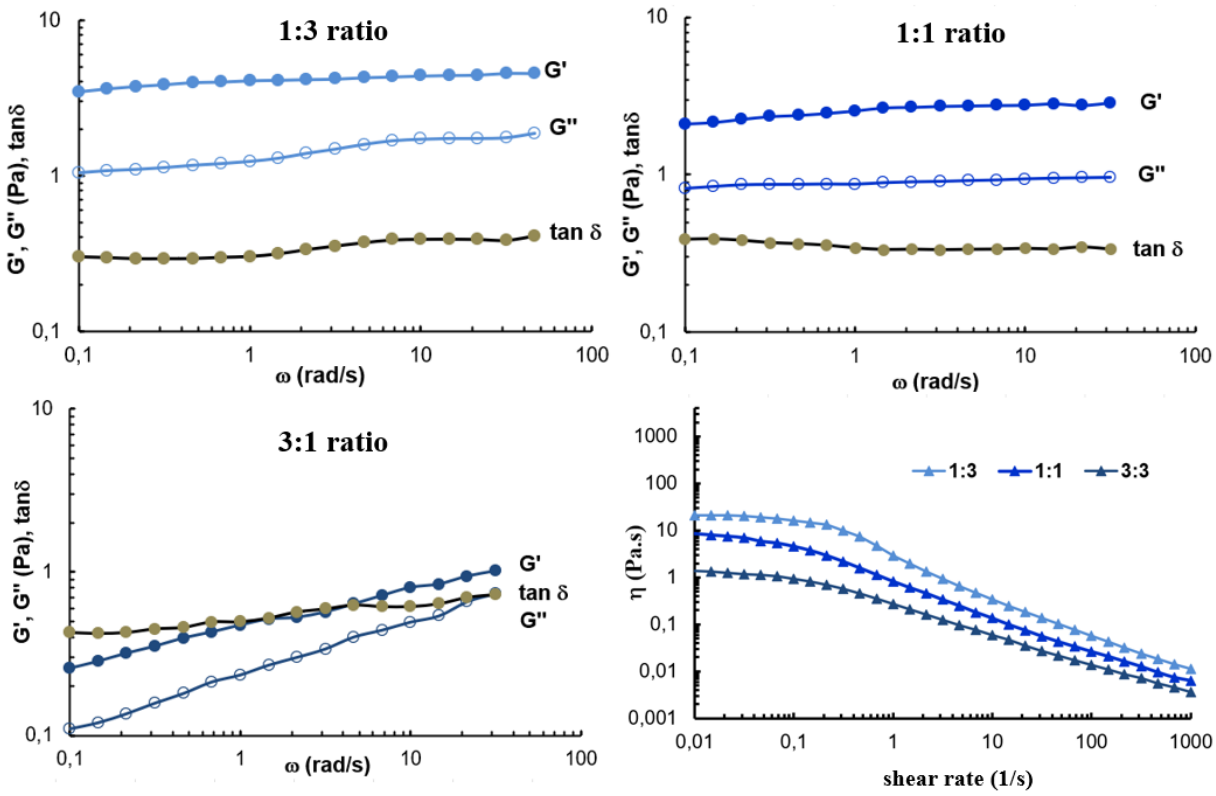


Figure 2.15. Viscoelastic parameters (G′,G″, tan δ) as a function of oscillation frequency at 37 °C and apparent viscosity as a function of shear rate.

2.4.4. Obtaining supramolecular gels based on lysine and a co-partner by using a polar aprotic solvent

For the preparation of ***S2***, ***S3*** and ***S4*** systems, Fmoc-Lys(Fmoc)-OH and a co-partner were used as: (i) **Fmoc-Serine-OH** to obtain S_2 system, (ii) **Fmoc-Glu** to obtain S_3 system and (iii) the **Fmoc-Gly-Gly-Gly-OH** tripeptide to obtain the S_4 system. Each system was co-assembled in three different ratios: 15:1, 5:1 and 2:1, starting from the minimum gelling concentration. Network formation and macroscopic structure was verified by the vial inversion test. Depending on the composition, translucent/transparent/opaque gels were obtained, with a stable structure that resists the vial inversion test.

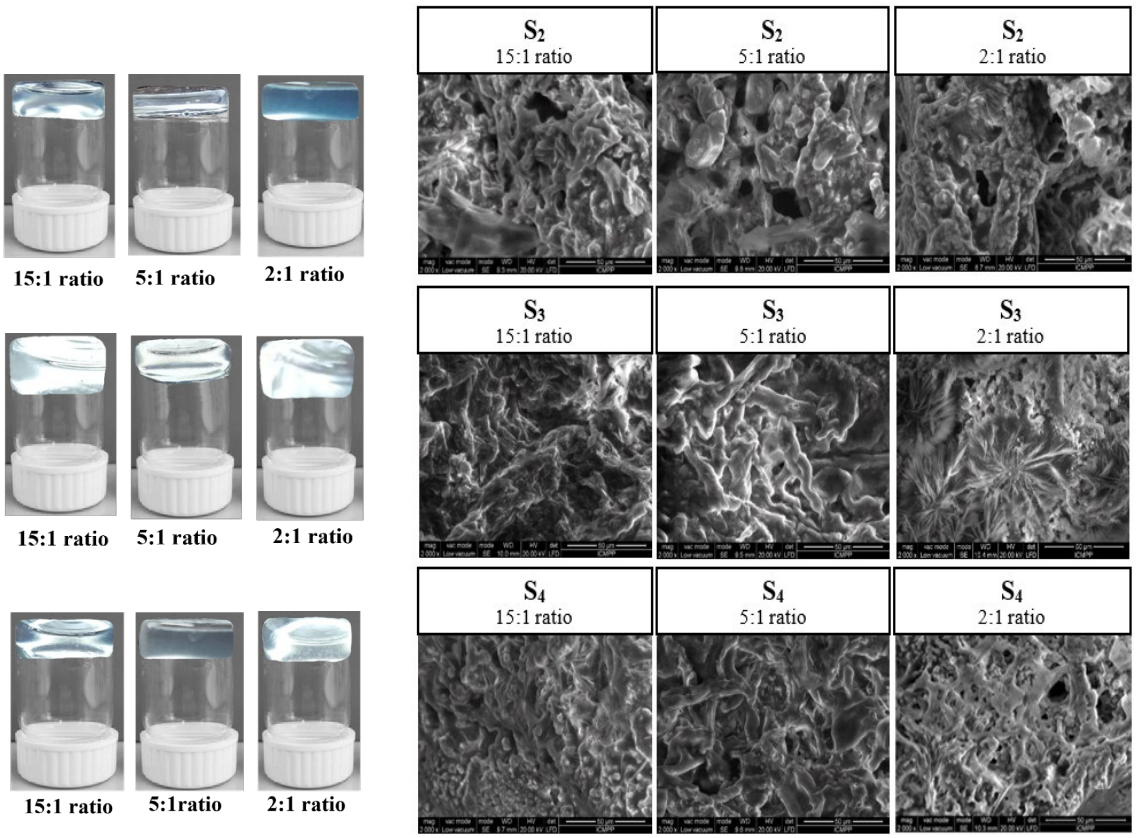


Figure 2.32. Appearance of S_2, S_3, S_4 co-assembled in 15:1, 5:1 and 2:1 ratio systems and their SEM images.

For all the co-assembled systems, the formation of fibers was demonstrated, with different morphologies depending on the chemical structure of the precursor compounds. In the case of the S*2* system, the fibers were well defined with an average diameter of 67±13 nm, slightly strangulated in some areas, forming a rather dense and compacted network, as seen in Figure 2.34. On the other hand, the S_3 system exhibits fibrillar formations arranged on a granular substrate, having an average width of 40±6 nm, as observed in the detailed AFM image. The S_4 system contains thick fibers with an average diameter of 114±15 nm and an apparent parallel arrangement at the studied scan level.

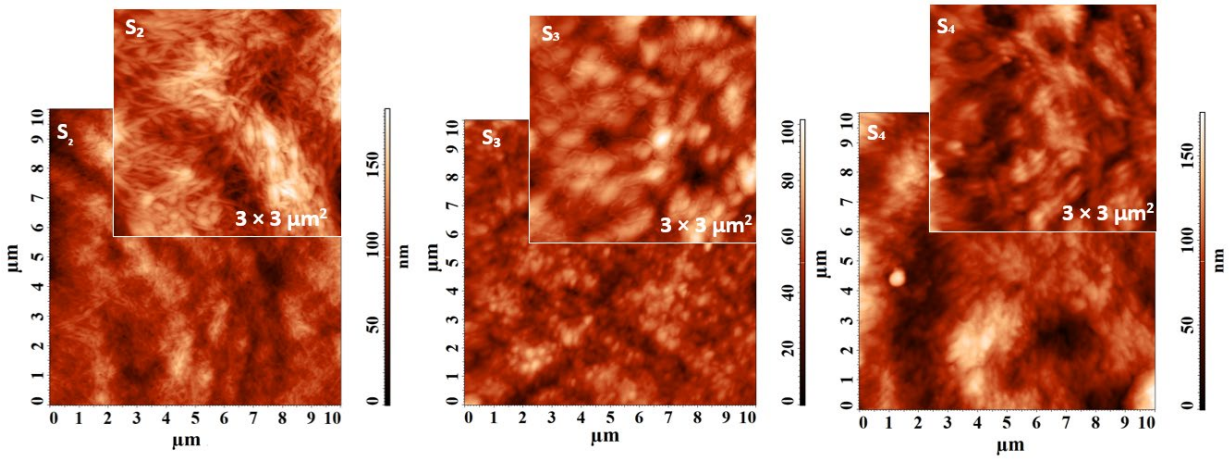


Figure 2.34. AFM images of the co-assembled systems (S_2, S_3 şi S_4) collected at 10×10 µm² and 3×3 µm².

From the rheological studies, it is noted that the systems co-assembled in a ratio of 5:1 present a gel behavior with G′> G″ and tan δ < 1. The value of the modulus of elasticity is influenced by the composition, so the S_3 system presents the modulus higher which confirms a better structuring and strength of the sample. Also, from the viscosity curves it is observed that the S_3 system is more structured, compared to the S_2 and S_4 gels.

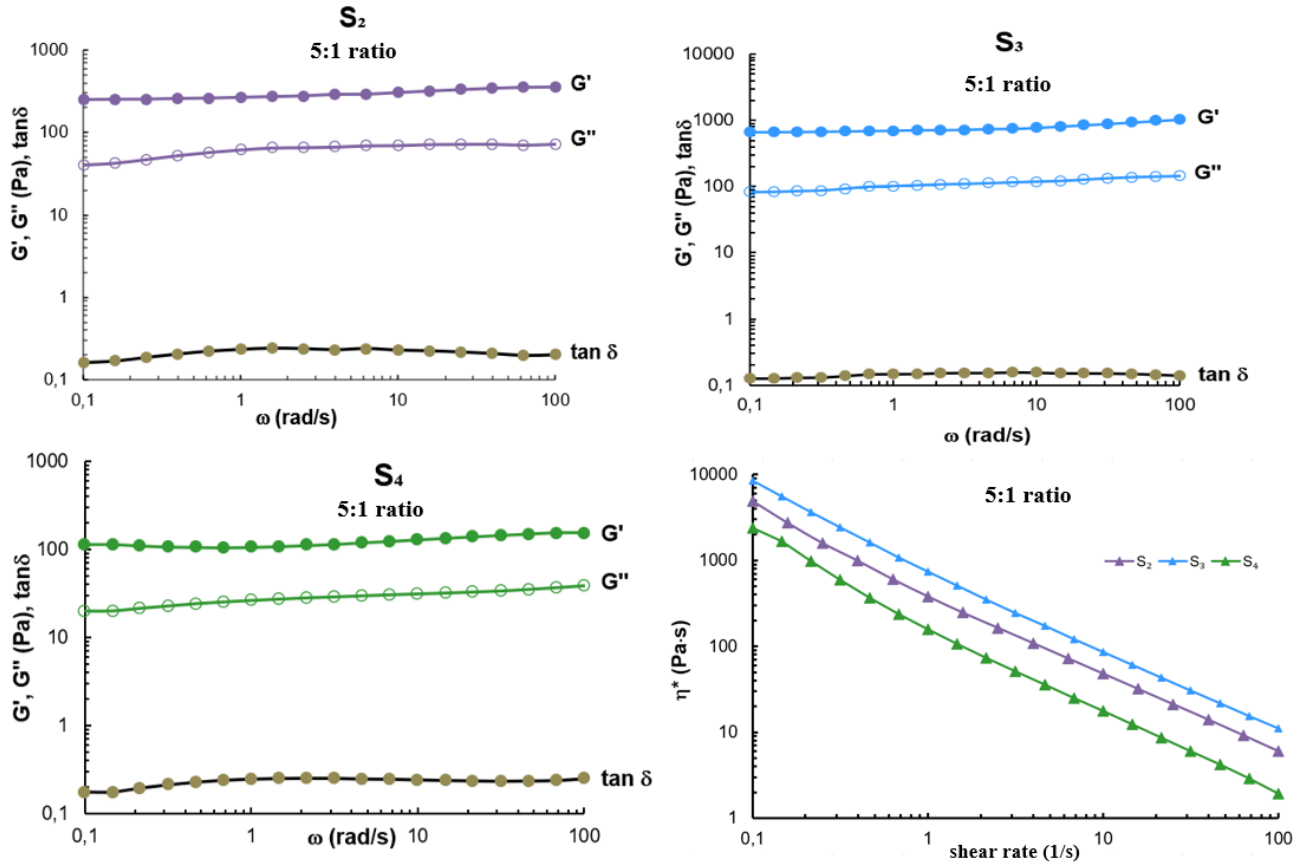


Figure 2.39. Viscoelastic parameters (G', G″ and tan δ) as a function of oscillation frequency (ω) for 5:1 ratio co-assembled gels and apparent viscosity as a function of shear rate.

This study demonstrated that Fmoc-modified amino acids and short peptides can be used as LMWGs for the formation of three-dimensional networks. Furthermore, supramolecular systems S_2, S_3, and S_4 exhibited varied architectures, even though the triggering factor was common. This aspect highlights that the presence of the polar aprotic solvent does not influence the mode of architectural organization.

CHAPTER III
MULTICOMPONENT HYDROGELS BASED ON SUPRAMOLECULAR STRUCTURES AND NATURAL POLYMERS

3.1. Objectives of the experimental study

The existing challenges in the design of hydrogel materials are oriented towards faithfully mimicking the 3D architecture of the ECM, as well as the dynamics of the mechanical and biochemical behavior of complex biological structures. Hydrogels based on polysaccharides and amino acid/peptide fragments are materials with promising properties due to their biocompatibility and biofunctionality. In addition, physically cross-linked networks are considered attractive supports due to their self-healing property, versatile chemical structure, and also easy fabrication process.

In this context, the aim of the study in this chapter is ***to design a new multicomponent system based on amino acids and natural polymers with stable structure, physical and chemical properties and applicability in the development of three-dimensional cell cultures***. The new hydrogels are considered superior to precursor compounds due to the way in which the biological properties of amino acids or peptides combine synergistically with the physicochemical properties of specific natural macromolecules.

3.2. The design of multicomponent gels

Based on good results obtained in the co-assembly step, the S_4 system based on Fmoc-Lys(Fmoc)-OH and Fmoc-Gly-Gly-Gly-OH was used to obtain and characterize multicomponent S_4/polymer systems. Starting from these considerations, the aim was to strengthen the architecture of the supramolecular gel and generate higher structures. The gelling polymers used in the study were gellan gum and agarose.

3.2.1. Obtaining multicomponent gels based on supramolecular structures and gellan gum

Gellan gum is a polysaccharide used in obtaining biomaterials due to both its mechanical and rheological behavior. The native form of gellan gum (with a high content of acyl groups) sold by SigmaAldrich® (Darmstadt, Germany) under the name Phytagel was used in the experimental study. Gellan gum will be found in sample coding as GG.

Obtaining the multicomponent gel based on supramolecular structures (S_4) and gellan gum was carried out in two stages. Initially, the S_4 supramolecular system was prepared in a 5:1 ratio, according

to the protocol presented in *Chapter II, Subchapter 2.4.4*, after which the gellan gum solution (2% w/v) was prepared in 0.01 M phosphate buffer (pH = 7.4). After complete dissolution of the gellan gum, the S_4 supramolecular co-assembly was added, obtaining the S_4_GG multicomponent system. The GG sample is considered the control sample of the multicomponent system.

As a formation principle, the S_4_GG multicomponent gel was obtained by co-assembling Fmoc-Lys(Fmoc)-OH with Fmoc-Gly-Gly-Gly-OH and rearranging the macromolecular chains of gellan gum as a result of their transition from a coiled conformation to double helix ("coil to double helix transition"), as represented in Figure 3.1

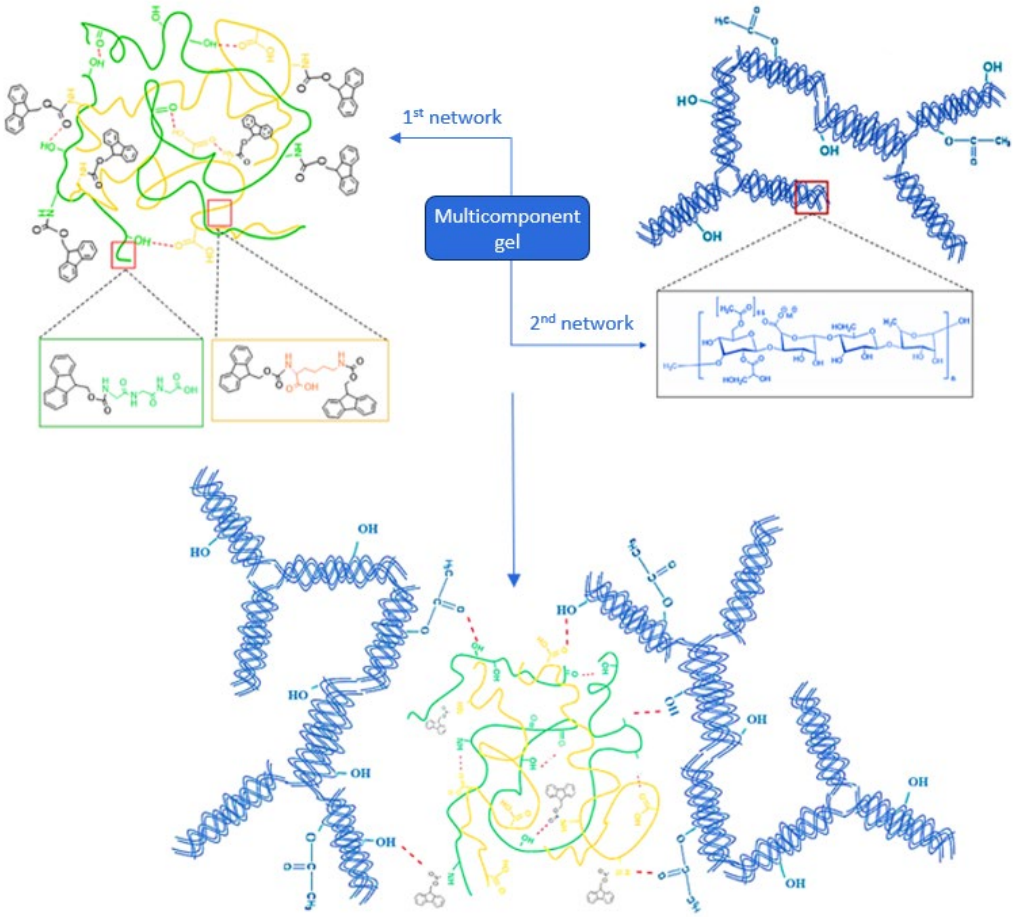


Figure 3.1. Schematic representation of the structure of the S_4_GG multicomponent hydrogel.

The S_4_GG multicomponent system was structurally characterized by Fourier transform spectroscopy (FTIR) which confirmed the interaction between the S_4 supramolecular system and gellan gum due to the formation of intra- and intermolecular hydrogen bonds. The thermal decomposition revealed that the introduction of gellan gum into the supramolecular system generates a more stable network, an aspect that can be determined by the presence of pyranose cycles in GG and at the same time with the greater number of secondary molecular interactions. Also, from the SEM images it was observed that by using the gum gellan together with S_4, the formation of a denser network with smaller pores occurs as a result of the formation of additional secondary molecular interactions.

Rheological studies revealed that by introducing gellan gum into the network of the co-assembled supramolecular system, the viscoelastic moduli increase from G' =~100 Pa and G" =~50 Pa (in the case of the S_4 system), to G' of approx. 850 Pa and G" of approximately 195 Pa (tan δ ≈ 0.229), an aspect that highlights the role of the polysaccharide in the final network. The rheological studies show that the systems obtained based on supramolecular structures and gellan gum present a behavior characteristic of gels: G'>G" and tan δ < 1.

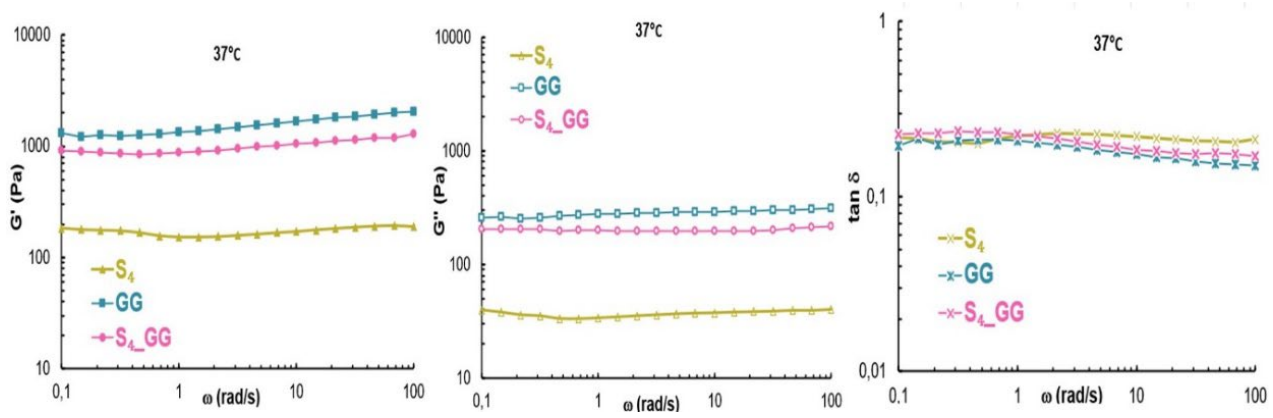


Figure 3.5. Dependence of G', G" and tan δ as a function of oscillation frequency for S_4, GG precursor networks and S_4_GG multicomponent gel at 37°C.

Also, the *in vitro* cytotoxicity studies show that the S_4_GG system is biocompatible and does not generate cytotoxic effects on fibroblasts, highlighting at the same time the importance of the supramolecular structure in the final system.

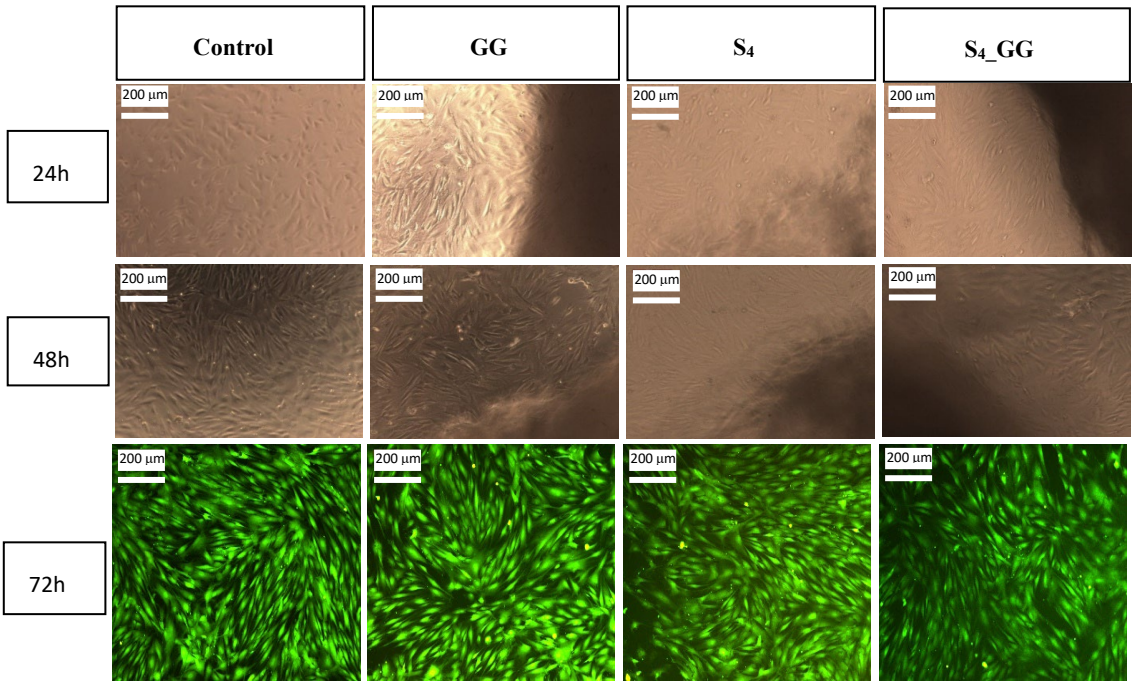


Figure 3.8. Phase-contrast cell cultures in contact with S_4, GG, and S_4_GG at 24 hours and 48 hours post-incubation and stained with Calcein AM at 72 hours post-incubation.

3.2.2. Obtaining multicomponent gels based on supramolecular structures and agarose

Agarose is a linear polysaccharide that allows gels to be obtained as a result of the heating (dissolving) – cooling (gelling) cycle. The agarose used in the experimental study was purchased from Sigma-Aldrich® (Darmstadt, Germany) and will be found in the sample coding as A. As in the case of obtaining the S_4_GG multicomponent system, the S_4_A was prepared in two steps. Initially, the S_4 supramolecular system co-assembled in a 5:1 ratio was obtained (procedure described in *subsection 2.4.4*), after which the 0.5% w/v agarose solution was prepared in 0.01 M phosphate buffer solution (pH = 7.4) under magnetic stirring at a temperature of 90ºC. The S_4 system was added over the agarose solution, under gentle stirring, thus obtaining the multicomponent gel S_4_A, and as a control sample, in the characterization stage, 0.5% w/v agarose was used.

The chemical structure of the multicomponent gel S_4_A obtained by the co-assembly of Fmoc-Lys(Fmoc)-OH with Fmoc-Gly-Gly-Gly-OH and the rearrangement of the macromolecular chains of A is illustrated in Figure 3.15.

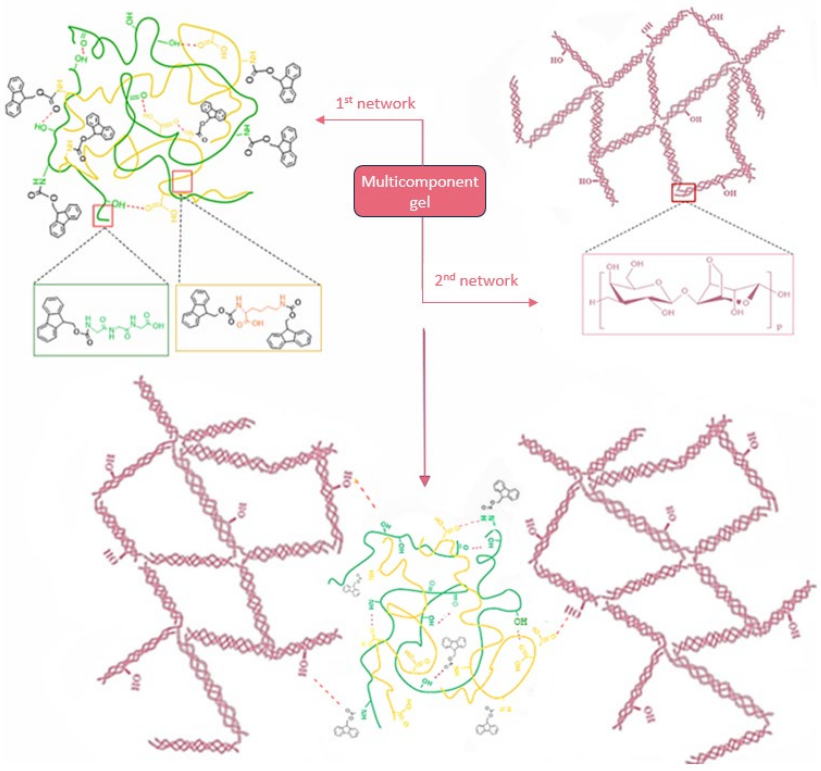


Figure 3.15. Schematic representation of the structure of the S_4_A multicomponent hydrogel

In the case of the S_4_A multicomponent system, structural characterization by Fourier transform spectroscopy (FTIR) confirmed the presence of functional groups both from the structure of the amino acid/tripeptide molecules and from the agarose structure. The recorded band shifts demonstrate the involvement of the -COOH and -NH2 groups in the interaction between the compounds and the formation of the three-dimensional network. The thermogravimetric analysis showed that the S_4_A system shows higher thermal stability compared to the precursor systems S4 and A. Furthermore,

rheological studies showed that the introduction of agarose into the co-assembled system S_4 generated an increase in the elastic modulus G' due to the additional bonds formed by S_4 and A.

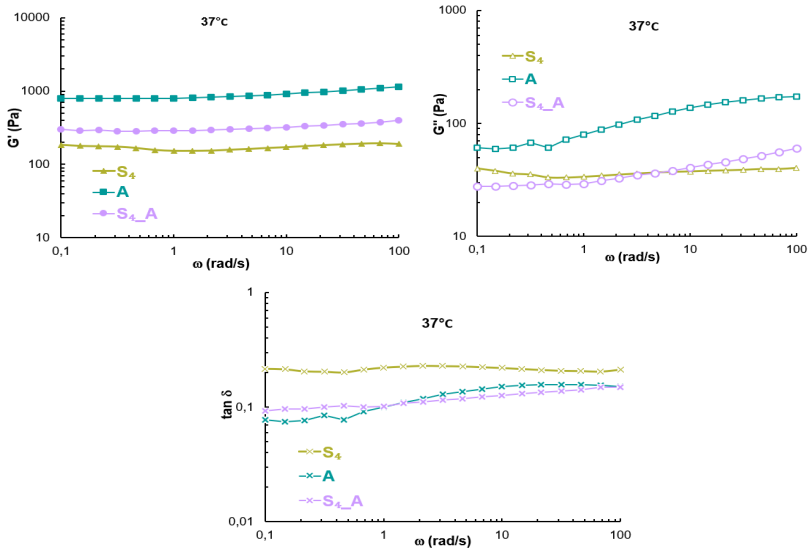


Figure 3.19. Dependence of G', G'' and tan δ as a function of oscillation frequency for S_4 and A precursor networks and S_4_A multicomponent gel at 37°C.

From cytotoxic point of view, S_4_A shows more than 90% cell viability at 72 hours of incubation with cells, and the results obtained from *in vivo* tests support the bioactive role that the supramolecular system has in the interaction between the material and the cell.

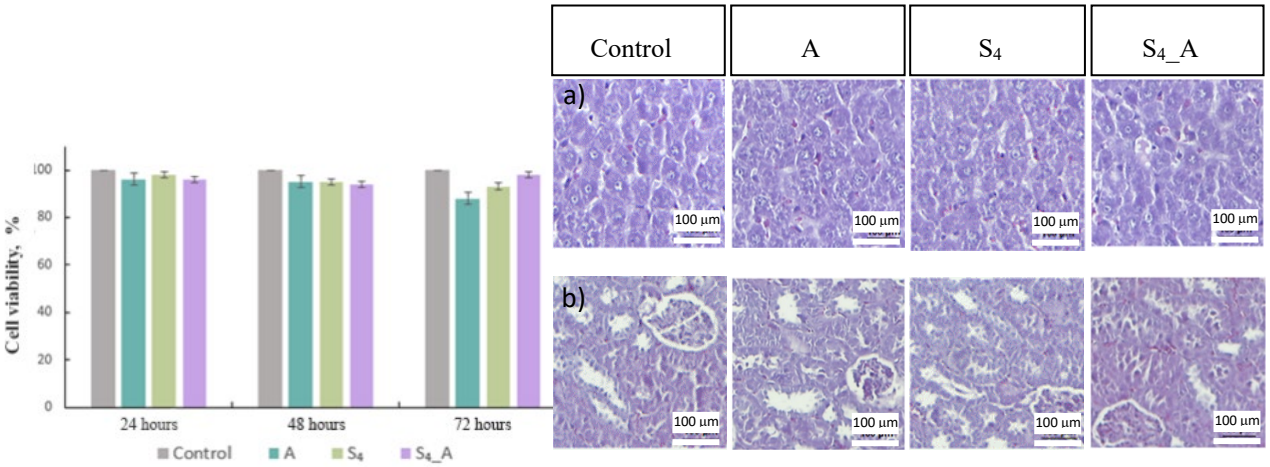


Figure 3.21. Cell viability determined by MTT assay at 24, 48 and 72 hours in contact with S_4, A and S_4_A materials (left) and optical microscopy images of a) liver structure and b) kidney structure following testing of S_4, A and S_4_A gels (right).

Corroboration of the results obtained from the performed analyzes support the possibility of using S_4_GG and S_4_A materials in biomedical applications.

CHAPTER IV
HYBRID GELS BASED ON SUPRAMOLECULAR STRUCTURES AND NATURAL AND SYNTHETIC

4.1. Objectives of the experimental study

The objective of the experimental study was to obtain hybrid systems based on amino acid/peptide sequences (S_4), natural polymers (sodium alginate - NaAlg) and synthetic polymers (poly(itaconic anhydride-co-3,9-divinyl-2, 4,8,10-tetraoxaspiro[5.5]undecane) – PItAU). The combination of these constituent chemical structures offers the possibility of generating **new hybrid materials with complex properties, capable of favoring the cell-matrix interaction.** The natural components have the role of maintaining the biocompatible character, but at the same time they are also recognized for the ability to form hydrogels. The synthetic component is representative, being a compound made within the collective and whose biocompatibility has been confirmed by *in vivo* studies.

The experimental study is based on three stages: (i) *the synthesis of the PItAU copolymer* followed by (ii) *its chemical modification by grafting it with a natural polymer (NaAlg),* optimizing the activity of the PItAU copolymer in the biological environment, and (iii) *the introduction of the S4 supramolecular system in the sodium alginate-based NaAlg/PITAU* hybrid network aimed at increasing the applicative potential in the biomedical field.

4.2. The design of the hybrid gels

The design of hybrid systems based on natural/synthetic polymers and supramolecular structures was carried out in three reproducible steps, "mentioned below".

4.2.1. Synthesis of the poly(itaconic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5] undecane) copolymer

According to the protocol developed by Diaconu et al. [143], the PItAU copolymer was synthesized by the radical polymerization process of itaconic anhydride (ItA) with 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane (U), in a 1:1.5 ratio between comonomers, in the presence of AIBN as initiator and 1,4-dioxane as solvent. The polymerization was carried out for 17 hours in an inert nitrogen atmosphere, at a constant temperature of 75 °C, with a stirring speed of 250 rpm. After cooling, the reaction mixture was precipitated dropwise into diethyl ether. Several washing steps with diethyl ether followed, after which the copolymer was dried in an oven at room temperature and 600

mm HG vacuum for 24 h. In Figure 4.1, the principle of the synthesis of the copolymer poly(itaconic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5] undecane) is schematically represented.

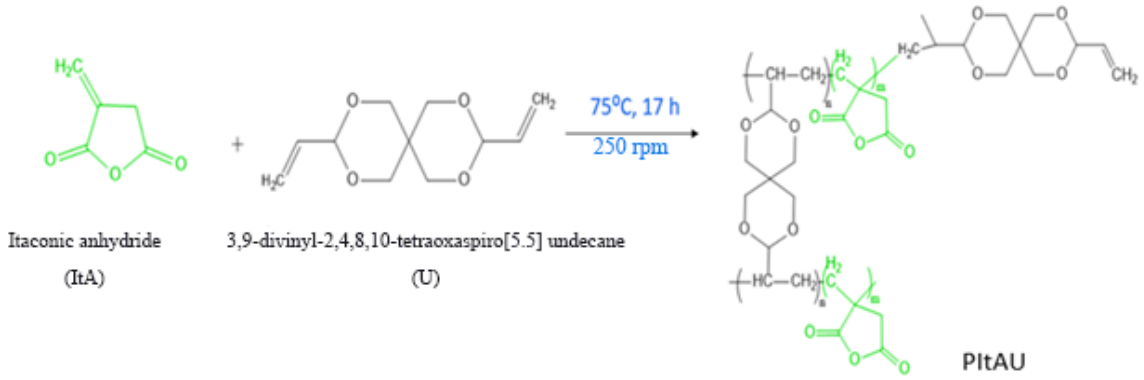


Figure 4.1. Schematic representation of PItAU copolymer synthesis.

Obtaining the copolymer was confirmed by the performed spectral analyzes (FTIR, ^{1}H-NMR), while the molecular mass was determined by static laser light scattering (SLS) which revealed that the molecular mass of the PItAU copolymer is 69.90 kDa.

4.2.2. Obtaining the NaAlg/PItAU bioconjugate

The second stage consisted in the formation of a hybrid system based on a mixture of natural polymer/synthetic polymer. The NaAlg/PItAU bioconjugate was obtained by mixing the NaAlg aqueous solution of concentration 30 mg mL^{-1} with an exact amount of copolymer (200 mg mL^{-1}) in dioxane solution to ensure a NaAlg:PItAU gravimetric ratio of 1: 3.5. The reaction between the natural polymer and the synthetic copolymer took place at room temperature in the absence of catalysts or cross-linking agents.

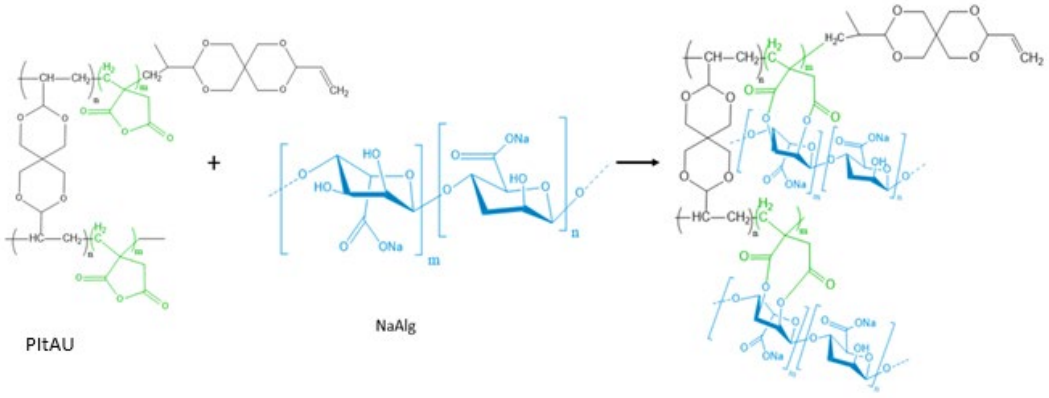


Figure 4.2. Schematic representation of obtaining the NaAlg/PItAU structure.

The FTIR spectrum of the NaAlg/PItAU bioconjugate shows specific bands that reveal the presence of both compounds and also the interaction between them. The presence of the band in the 3495 cm^{-1} region is attributed to the stretching vibrations of the O-H group in the NaAlg structure, while the disappearance of the bands in the 1862 cm^{-1} – 1782 cm^{-1} region confirms the interaction between NaAlg and PItAU, as a result of the itaconic anhydride ring opening and the formation of

new intermolecular bonds. Also, the bands in the region 1213 cm^{-1} - 1170 cm^{-1} confirm the presence of the spiroacetal fragments characteristic of the PItAU copolymer.

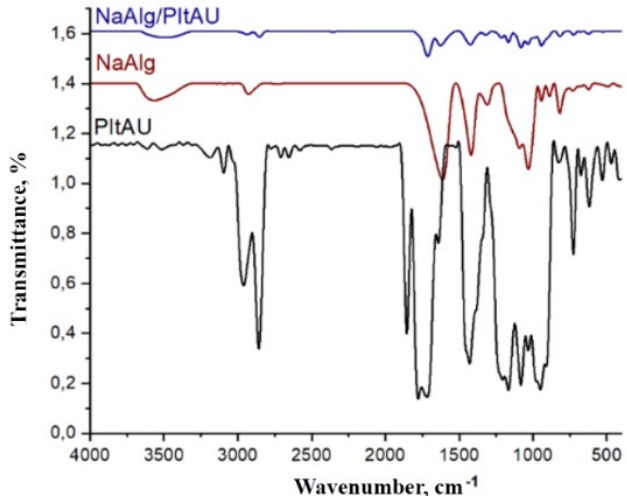
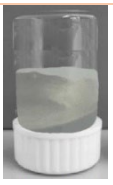


Figure 4.5. FTIR spectra of NaAlg/PItAU, NaAlg and PItAU.

4.2.1. Obtaining hybrid gels based on supramolecular structures and NaAlg/PItAU

Obtaining hydrogels based on NaAlg/PItAU and co-assembled supramolecular structures was carried out in two stages: the first stage aimed at obtaining the NaAlg/PItAU bioconjugate, and the second stage focused on the preparation of solutions of Fmoc-Lys(Fmoc)-OH in a concentration of 0.5% w/v and Fmoc-Gly-Gly-Gly-OH of 0.1% w/v, according to the protocol described in *subchapter 2.4.4.* After performing the two steps, the mixtures based on NaAlg/PItAU and S_4 (ratio 15:1, 5:1 and 2:1) were left at room temperature for 24 h for maturation. Their appearance after the vial test inversion is presented in table 4.3.

Table 4.3. Appearance of mixtures based on NaAlg/PItAU and S_4 (ratio 15:1, 5:1 and 2:1).

Sample appearance		
NaAlg/PItAU_($S_{4\ 15:1}$)	NaAlg/PItAU_($S_{4\ 5:1}$)	NaAlg/PItAU_($S_{4\ 2:1}$)
		

Taking into account that no stable gel was formed after 24 hours, an attempt was made to prepare solutions of Fmoc-Lys(Fmoc)-OH and Fmoc-Gly-Gly-Gly-OH in a concentration of 0.5% w/v, subsequently being mixed in 1:1 and 1:3 gravimetric ratio. Also, the ratio between the NaAlg/PItAU structure and the S_4 system is 1:1 v/v.

In Table 4.5. the appearance of both control and hybrid gels following the vial inversion test can be observed. The samples have an opaque appearance, with slight differences in consistency. As can be noted, the control samples NaAlg/PItAU_M_2 and NaAlg/PItAU_M_5 are unstable showing slight flow.

The sample containing NaAlg/PItAU and Fmoc-Lys(Fmoc)-OH (M_2) will be found in the study under the name NaAlg/PItAU_M_2, and the one containing NaAlg/PItAU and Fmoc-Gly-Gly-Gly-OH (M_5) will be NaAlg/PItAU_M_5. These are considered as control samples of NaAlg/PItAU_(S_4 1:1) and NaAlg/PItAU_(S_4 1:3) hybrid systems.

Table 4.5. Appearance of NaAlg/PItAU_(S_4 1:1) and NaAlg/PItAU_(S_4 1:3) hybrid gels and NaAlg/PItAU_M_2, NaAlg/PItAU_M_5 controls after the vial test inversion.

Appearance of hybrid gels					
S_4 1:1 ratio	S_4 1:3 ratio	NaAlg/PItAU_M_2	NaAlg/PItAU_M_5	NaAlg/PItAU_(S_4 1:1)	NaAlg/PItAU_(S_4 1:3)
					

From the FTIR spectra of the hybrid systems it was observed that the presence of the tripeptide in a higher ratio leads to the formation of a greater number of hydrogen bonds. This aspect is also confirmed by the thermal analysis, which shows that the NaAlg/PItAU_(S_4 1:3) gel is more stable compared to the NaAlg/PItAU_(S_4 1:1).

Furthermore, the water vapor sorption capacity was evaluated in dynamic mode, and the obtained sorption/desorption isotherms can be associated with Type IV isotherms. This type of isotherm with hysteresis is characteristic of porous surfaces, being specific for a hydrophilic material. Also, the BET data obtained from the evaluation of the water sorption/desorption behavior demonstrate that the NaAlg/PItAU_(S_4 1:1) and NaAlg/PItAU_(S_4 1:3) hybrid networks exhibit pores with an average size of 2 nm and specific surface areas with values ranging from 280 to 340 m²/g.

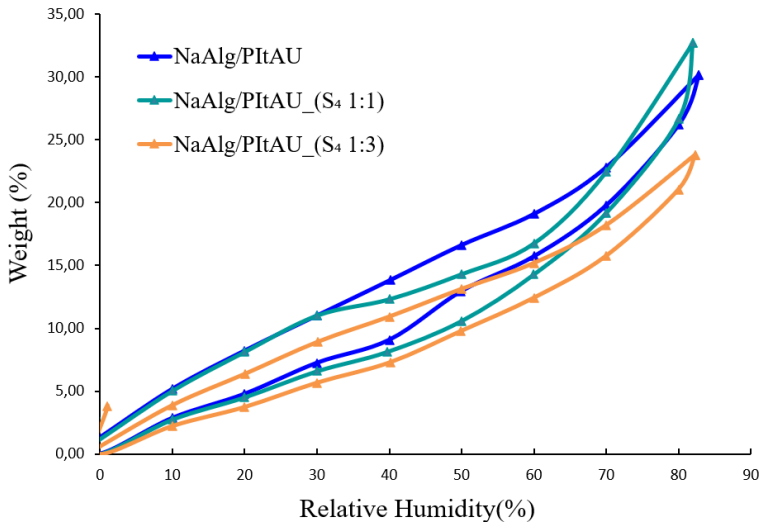


Figure 4.17. Sorption/desorption isotherms of NaAlg/PItAU, NaAlg/PItAU_(S_4 1:1) and NaAlg/PItAU_(S_4 1:3) samples

The synergistic effects generated by the combination of natural amino acid/tripeptide fragments and NaAlg with the synthetic polymer PItAU led to obtaining the NaAlg/PItAU_(S_4 1:3) hybrid system with superior properties to the initial compounds.

CHAPTER V
DOUBLE NETWORK GELS BASED ON POLY[2-(DIMETHYLAMINO)ETHYL METHACRYLATE] AND SUPRAMOLECULAR STRUCTURES

5.1. Objectives of experimental study

The objective of this study was the design of double-network hydrogels made from a first network formed by chemical cross-linking of PDMAEMA chains with N-N'-methylene-bis-acrylamide and interpenetrated with the second supramolecular network formed by co-assembly of the amino acid Fmoc-Lys(Fmoc)-OH with the tripeptide Fmoc-Gly-Gly-Gly-OH. The interpenetration of the two networks offers the advantages of obtaining soft and tough hydrogels that intrinsically possess mechanical resistance due to their contrasting properties. The amphoteric structural units were chosen due to their ability to interact and generate double network systems with specific physicochemical properties.

5.2. *"In situ"* preparation of double network gels based on poly(2-(dimethylamino)ethyl methacrylate) and supramolecular structures

The *"in situ"* formation of double networks (DN) was achieved by obtaining the synthetic network based on PDMAEMA that interpenetrates with the supramolecular one generated by the co-assembly of the Fmoc-Lys(Fmoc)-OH and Fmoc-Gly-Gly- Gly-OH co-partners.

The PDMAEMA-based network was prepared by radical polymerization of the 2-(dimethylamino)ethyl methacrylate monomer in the presence of the ammonium persulfate (APS)/N,N,N',N'-tetramethyl-ethylenediamine (TEMED) redox initiation system, using water as a reaction medium and the cross-linking of the polymer chains with N,N'-methylene-bis-acrylamide, thus obtaining the PDMAEMA synthetic network. The volumetric ratio of APS to TEMED was 10:1.

Figure 5.1 shows the principle of PDMAEMA network formation. Following the polymerization process, the poly (2-(dimethylamino)ethyl methacrylate) polymer is formed which is subsequently cross-linked with N,N'-methylene-bis-acrylamide. The presence of vinyl groups (-$CH{=}CH_2$) in the bisacrylamide structure determines the formation of covalent bonds with the functional groups of PDMAEMA, thus establishing interactions between the polymer chains.

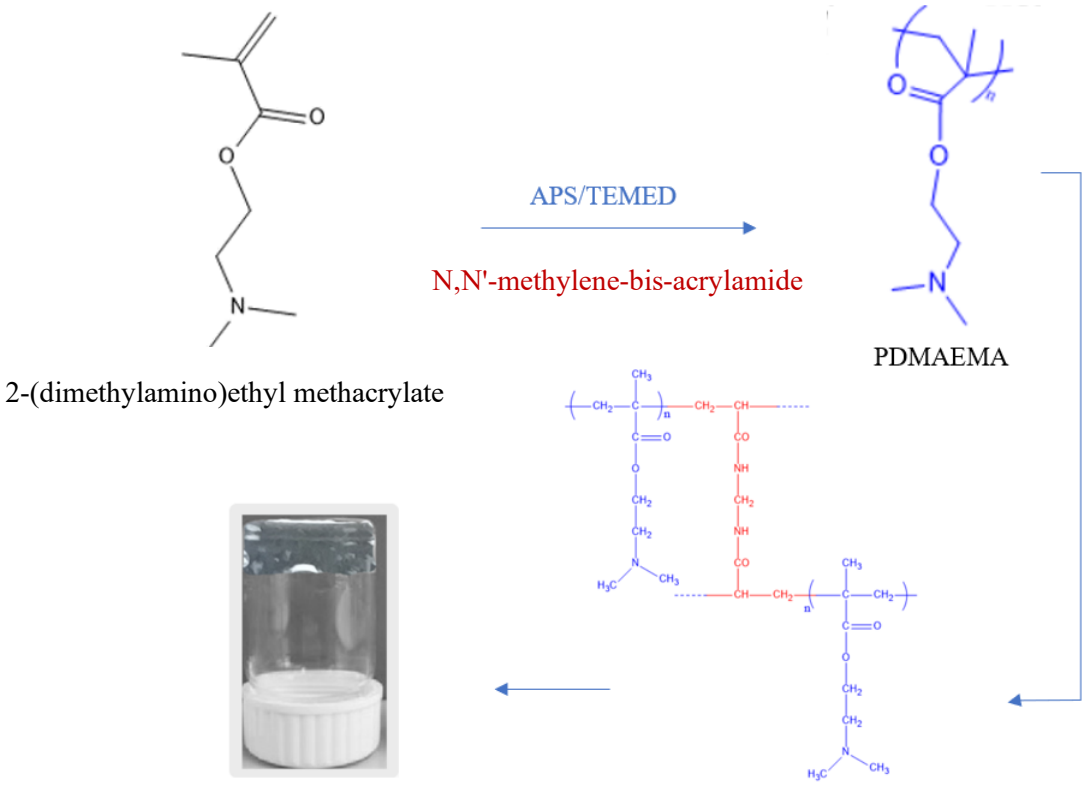


Figure 5.1. Schematic representation of the PDMAEMA-based synthetic network.

The preparation of the supramolecular network S_4 was carried out according to the protocol presented in *subchapter 2.4.4.*, specifying that, in the present study, the co-partners Fmoc-Lys(Fmoc)-OH (M_2) and Fmoc-Gly-Gly-Gly-OH (M_5) were co-assembled in 1:1 and 1:3 ratios as a result of increasing the tripeptide concentration from 0.1% w/v (originally used in the formation of the co-assembled S_4 system in 15:1, 5:1 and 2:1) at 0.5% w/v. This increase in concentration is due to the fact that PDMAEMA_(S_4 15:1), PDMAEMA_(S_4 5:1) and PDMAEMA_(S_4 2:1) systems do not form stable gel. Therefore, PDMAEMA_(S_4 1:1) and PDMAEMA_(S_4 1:3) systems were obtained.

Table 5.1. Appearance of PDMAEMA_(S_4 1:1) and PDMAEMA_(S_4 1:3) double network gels and PDMAEMA_M_2, PDMAEMA_M_5 controls after vial test inversion.

Sample appearance			
Control samples		**DN gels**	
PDMAEMA_M_2	PDMAEMA_M_5	PDMAEMA_(S_4 1:1)	PDMAEMA_(S_4 1:3)
			

From the study of the swelling behavior, a sudden increase in the degree of swelling is noted in the first five minutes, a behavior that is due to the hydrophilic character of the PDMAEMA polymer [247]. The PDMAEMA_(S_4 1:3) DN system shows a higher degree of swelling than PDMAEMA_(S_4

1:1) due to the morphology with uniformly distributed pores, which play an active role in the diffusion of phosphate buffer solution molecules in the gel matrix. Also, the presence of the M_5 precursor in the PDMAEMA_M_5 system causes a greater increase in the degree of swelling, compared to M_2, as a result of the formation of a greater number of H bonds between -CO-NH- and the environment.

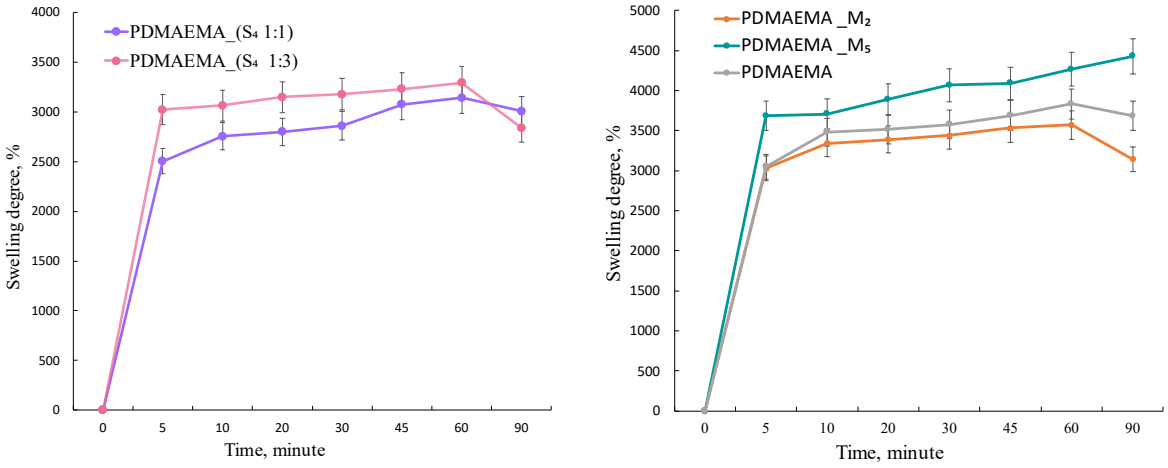


Figure 5.8. Kinetics of the swelling degree of the PDMAEMA_(S_4 1:1), PDMAEMA_(S_4 1:3) DN systems and the PDMAEMA, PDMAEMA_M_2 și PDMAEMA_M_5 control samples.

The biological tests showed the absence of significant changes in the hematological, biochemical or immunological parameters investigated, an aspect that suggests a good hemocompatibility *in vitro* and biocompatibility *in vivo* in mice.

The results obtained from the experimental study demonstrate that the interpenetration of the PDMAEMA network with the S_4 (1:1, 1:3) supramolecular network led to the formation of double network gels: PDMAEMA_(S_4 1:1) and PDMAEMA_(S_4 1:3).

CHAPTER VII
GENERAL CONCLUSION AND PERSPECTIVES

In recent years there has been immense interest in studying supramolecular gels derived from low-molecular-weight gelators (LMWGs). The faithful mimicry of self-assembled supramolecular structures inspired by nature has become an increasingly attractive research direction due to individual molecules exhibiting the ability to generate a range of nano/micro-aggregates, such as fibers, sheets, tubes, spheres, α-helices, vesicles. The diversity of structural architectures is based on the aggregation of molecules through different non-covalent interactions (hydrogen bonds, π–π interactions, van der Waals interactions, hydrophobic interactions).

Currently, research in the field is opening new paths for the use of supramolecular gels as soft functional materials, aiming at potential applications in medicine and other related sciences. Amino acids and peptides have attracted the attention of researchers due to their prevalence in living systems, offering new perspectives in the exploitation of supramolecular gels as 3D supports for the growth and development of cell cultures. However, in addition to the structural advantages and chemical versatility of this type of molecules, there are also disadvantages generated by the reversible and dynamic nature of non-covalent bonds. Therefore, the use of LMWGs alongside natural/synthetic polymers leads to obtaining multicomponent gels with intrinsic properties superior to the precursor compounds.

The doctoral thesis entitled "***Multicomponent gels: modeling structures with low-molecular-weight gelators"*** was structured in two parts: Part I (Chapter I) presents a literature study regarding the directions addressed in the thesis, and Part II presents the original contributions (Chapter II -V) and the techniques used (Chapter VI). The thesis ends with a series of general conclusions (Chapter VII).

The original results from Part II, structured in four chapters, targeted the following research directions:

- development of supramolecular systems in the form of gels based on amino acids and short peptides used as LMWGs;
- obtaining and characterizing multicomponent gels by including natural gelling polymers in the supramolecular matrix and investigating the applicative potential as a 3D matrix for the growth and development of cell cultures;
- the preparation and characterization of hybrid gels based on synthetic/natural polymers and supramolecular structures in order to synergistically combine the physico-chemical and biological properties of the precursor compounds;

- the synthesis and characterization of semi-IPN type double network (DN) gels based on the development of the polymer matrix and the interpenetration of supramolecular structures.

The following aspects can be concluded from the realised studies:

SUPRAMOLECULAR GELS BASED ON AMINO ACIDS AND PEPTIDES AS LOW MOLECULAR WEIGHT GELATORS

- Following the self/co-assembly study, four supramolecular systems were obtained starting from the use of two external stimuli: *pH change* (S_1) and *the use of a polar aprotic solvent* (S_2, S_3, S_4). Tryptophan and lysine-based S_1 system was co-assembled in 1:3, 1:1 and 3:1 ratio as a result of 0.5% minimum gelation concentration (CMG) for both co-partners of the system. In the case of the S_2, S_3, S_4 systems, the common element was lysine which was co-assembled with serine, glutamic acid and the glycyl-glycyl-glycine tripeptide. The ratio of the compounds was 15:1, 5:1 and 2:1, as a result of 0.5% the CMG for lysine and 0.1% for the co-partner used, providing information on the contribution of each compound to the network properties finals;
- The organization/structuring capacity of the compounds was confirmed by the analyzes performed on the systems in the solution state, showing that S_1 presents a possible helical structural arrangement generated by π–π interactions of the fluorenyl fragments in the Fmoc structure and the indole rings in the tryptophan structure, and intra- and intermolecular hydrogen bonds were the dominant driving forces of the S_2, S_3 and S_4 systems;
- In the case of the S_2, S_3 and S_4 systems, molecular aggregation occurs at different time intervals, depending on the co-partner of the lysine. When using Fmoc-Gly-Gly-Gly-OH the co-assembly time is ~ 30 minutes, while the presence of Fmoc-serine-OH allows the association/assembly point to appear after 90 minutes. The S_3 system which is based on the interaction of Fmoc–Lys(Fmoc)-OH with Fmoc-glutamic acid is formed after 24 hours;
- *X-ray diffraction* studies show that, following the co-assembly process, supramolecular systems with a more ordered molecular arrangement are obtained, compared to the molecular arrangement of the precursors;
- *Fourier transform infrared spectroscopy (FTIR) studies* reveal that as the amount of Fmoc–Lys(Fmoc)-OH increases, a greater number of -CO-NH- groups is involved in the formation of intra- and intermolecular bonds of hydrogen. The S_2, S_3 and S_4 supramolecular systems show band shifts that attest to the formation of intra- and intermolecular physical bonds and that ensure their organization in a 3D network;

- Also, the high thermal stability of the co-assembled S_1 system in the 1:3 ratio (Fmoc-Trp-OH:Fmoc-Lys(Fmoc)-OH) is correlated with the density of the Fmoc aromatic groups and the incomplete decomposition of the fluorenyl rings. The thermal analysis showed that the S_4 system has a higher thermal stability than the other analyzed systems;
- The complex *morphological study* confirms that the amino acids and the short peptide used as LMMGs initially aggregate in the form of dendrimer, spherulites, fibrils, vesicles that later ensure the formation of fibers and their organization in three-dimensional networks. Depending on the co-partners used, the co-assembled systems have a different appearance: S_1 shows fibrillar morphology, S_2 shows straight fibers aligned side by side, while S_3 has a less dense network with shorter and more flexible fibers, and the system S_4 features a much denser network of branch points;
- The *rheological study* revealed that the obtained supramolecular systems present a gel-type behavior ($G' > G''$ and $\tan \delta < 1$), an aspect that supports the ability of amino acids and short peptides to act as LMMGs in the studied systems. In the case of the S_1 system co-assembled in a 3:1 ratio, a decrease in the viscoelastic modulus is noted, along with the increase in the content of Fmoc-Trp-OH. Thus, the indole rings create space between the molecules and diminish their potential to form molecular interactions;
- The cytocompatible character of the analyzed systems was determined by *in vitro* and *in vivo* tests, after the intraperitoneal administration of material fragments in mice. The lack of variation in liver enzyme activity testified that the materials are biocompatible, with no negative influence of the inflammatory process on liver function.

The supramolecular gels obtained and characterized in this study show optimal characteristics for use in medical applications. Due to its properties, the S4 system was chosen for use in the following studies.

MULTICOMPONENT HYDROGELS BASED ON SUPRAMOLECULAR STRUCTURES AND NATURAL POLYMERS

- The inclusion of natural polymers in the S_4 supramolecular system has been another research direction aimed at strengthening the gel architecture and generating rheologically superior structures based on additional physical interactions;
- The use of *gellan gum* and *agarose* is justified by their ability to gel as a result of the rearrangement of macromolecular chains induced by temperature variation;
- The *FTIR structural analysis* confirms that the two multicomponent systems (S_4_GG and S_4_A) are based on intra- and intermolecular physical bonds, and the *thermogravimetric analysis* highlights the thermal behavior and the changes generated by the introduction of macromolecules, in both cases there is an increase in thermal stability;

- *Morphological studies* indicate the formation of homogeneous porous networks in direct correlation with their composition;
- The *viscoelastic behavior* of multicomponent gels was studied through rheological measurements which showed that the presence of macromolecules in the S_4 system significantly contributes to the increase of the storage modulus, from ~300 Pa (S_4) to 500 Pa (S_4_A) or ~1000 Pa (S_4_GG);
- The materials were tested from the point of view of *bioadhesion properties*, using hydrated dialysis membranes as an *in vitro* tissue model. The obtained results suggest that the introduction of gellan gum into the supramolecular matrix increases the adhesion force and the mechanical work of adhesion of the S_4_GG material due to the increase in the number of hydrogen bonds between the material and the membrane. In the case of the S_4_A system, a decrease in the bioadhesion force and the mechanical work of adhesion is observed to values lower than those of the S_4 supramolecular network. The nonionic character of agarose is the factor responsible for the low adhesive properties of sample A;
- *In vitro* biocompatibility testing was performed on primary fibroblasts confirming that the tested materials do not show cytotoxicity. In the case of both systems, a satisfactory cell viability is noted, even after 72 hours of incubation, but the cell viability obtained for the **S_4_A gel demonstrates its potential to be used as a platform for cell growth and proliferation as a result of increased cell viability concomitant with incubation period;**
- *In vivo* biocompatibility testing of the gels was carried out by determining the hematological, immunological and histopathological profile of Wistar rats, demonstrating that the materials did not generate significant variations on the serum values of biological parameters and leukocyte formula elements. The histopathological examination revealed that the materials did not influence the architecture of the hepatocytes or the conformation of the medullary kidney.

The corroboration of the obtained results following the analyzes carried out support the possibility of using the multicomponent systems S_4_GG and S_4_A in biomedical applications, as a platform for cell growth and proliferation as a result of the increase in cell viability simultaneously with the incubation period.

HYBRID GELS BASED ON SUPRAMOLECULAR STRUCTURES AND NATURAL AND SYNTHETIC

- The poly(maleic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane) synthetic copolymer used in the study was obtained by the radical polymerization process, followed by its chemical modification by grafting on sodium alginate, in order to optimize the activity of the copolymer in the biological environment;

- The structure of the copolymer was confirmed by *FTIR, ^{1}H-NMR spectral analyses*, and the grafting reaction was highlighted by FTIR spectroscopy as a result of the opening of the anhydride cycle characteristic of itaconic anhydride and the formation of an ester bond;
- *FTIR structural analysis* of the hybrid systems obtained following the introduction of the S_4 supramolecular structure in the NaAlg/PItAU network attests to the presence and interaction of the compounds through the formation of intra- and intermolecular physical bonds;
- *TG/DTG analysis* showed that the presence of NaAlg/PItAU in the S_4 system contributes to the thermal stability, with small variations given by the ratio between the co-partners of the S_4 system, an aspect also observed from the *SEM microscopy*. The presence of the tripeptide in a higher ratio (S_4 1:3) leads to obtaining a homogeneous network with a porous appearance as a result of a greater number of molecular interactions generated by the -CO-NH- group;
- From the *rheological studies*, it appears that the NaAlg/PItAU_(S_4 1:3) hybrid system exhibits behavior characteristic of gels (G' > G″ and tan δ<1), and the G' modulus value higher than that of the NaAlg/PItAU system confirms that the secondary molecular interactions formed between (S_4 1:3) and NaAlg/PItAU are responsible for increasing the dynamic stiffness;
- The *evaluation of the fluid absorption capacity* was carried out in an environment with phosphate buffer solution with pH 7.4, favorable for cell cultures. The equilibrium state was reached after 96 hours, the gels showing a maximum degree of swelling between 3000 and 6700%, an aspect that gives them a superabsorbent character;
- The *vapor sorption capacity* of water was evaluated in dynamic mode, and the obtained sorption/desorption isotherms are associated with type IV isotherms characteristic of porous surfaces and specific for a hydrophilic material. Furthermore, BET data confirms that the materials exhibit an average pore size of 2 nm and specific surface areas ranging from 280 to 340 m²/g;
- *In vitro* biocompatibility tests indicate a good interaction between materials and cells, with cell viability values of over 75% recorded after 96 hours of incubation.

The synergistic effects obtained following the combination of amino acid/tripeptide type fragments with NaAlg and the synthetic polymer PItAU led to obtaining the NaAlg/PItAU_(S_4 1:3) hybrid system with superior properties to the initial compounds.

DOUBLE NETWORK GELS BASED ON POLY(2-(DIMETHYLAMINO)ETHYL METHACRYLATE) AND SUPRAMOLECULAR STRUCTURES

- During the study, 5 samples were prepared and studied, one of which was the pure PDMAEMA network, two were networks based on PDMAEMA and Fmoc-Lys(Fmoc)-OH, respectively Fmoc-Gly-Gly-Gly-OH, used as controls and two were the double network gels containing PDMAEMA and S_4 in 1:1 ratio and S_4 in 1:3 ratio type supramolecular structures;

- The PDMAEMA synthetic network, considered the first network in the structure of DN gels, was obtained by the radical polymerization of 2-(dimethylamino)ethyl methacrylate in the presence of the APS/TEMED initiator system and the crosslinking of the polymer chains with N-N'-methylene-bis- acrylamide;
- DN gels obtaining was based on the interpenetration of the second network, represented by the S_4 supramolecular system, in the polymer matrix;
- The first network is responsible for stability and mechanical strength, while the second network provides flexibility due to the secondary molecular interactions formed between co-partners;

Following the analyzes carried out, it can be concluded that the synergistic effects of the two networks provided superior physico-chemical and biological properties to the precursor compounds.

Perspectives

As perspectives, the research directions developed within the doctoral thesis will be expanded and oriented towards:

- in-depth studies on the assembly of peptide components (protonation-deprotonation of amino groups and carboxylic groups by potentiometric titration;
- studies on the stability of hydrogel-type systems by exposure to simulated solutions/body fluids;
- assessment of *in vitro* and *in vivo* enzymatic biodegradability*;*
- an evaluation of the "*in situ"* encapsulation capacity of cells in the structure of supramolecular systems;
- optimization of gel systems based on short peptides for various therapeutic areas;

As a general perspective, the development of dynamic, rapid, feasible and sustainable acquisition/synthesis techniques that provide the possibility to make additional changes on the amino acid chain, the N- or C- terminus or at the level of the side chains is emerging. To faithfully mimic the extracellular matrix, stabilization of secondary structures obtained by amino acid/peptide co-assembly is required to generate promising therapeutic platforms.

Dissemination of results and scientific activity

The original results obtained and presented in the doctoral thesis are the subject of five scientific articles, two communications and three posters:

- ***Published scientific articles***

1. **Alexandra Croitoriu**, Loredana E. Nita, Alina G. Rusu, Florica Doroftei, Liliana Verestiuc, Co-assembled peptides hierarchically oriented for supramolecular gel formation, Rev. Roum. Chim., 2021, 66(5), 449–458. doi: 10.33224/rrch.2021.66.5.08 (IF 0,5)

2. **Alexandra Croitoriu**, Loredana Elena Nită, Aurica P. Chiriac, Alina Gabriela Rusu, Maria Bercea, New Physical Hydrogels Based on Co-Assembling of FMOC–Amino Acids. *Gels*. 2021; 7(4):208. doi:10.3390/gels7040208 (IF 4,6)
3. **Alexandra Croitoriu**, Alina Gabriela Rusu, Alina Ghilan, Maria Bercea, Loredana Elena Nita, Aurica P. Chiriac, New Fmoc-amino acids/peptides-based supramolecular gels obtained through co-assembly process: preparation and characterization, *Polymers,* 2022, *14*(16), 3354. doi:10.3390/polym14163354 (IF 5,0)
4. Loredana Elena Nita, **Alexandra Croitoriu**, Alexandru M. Serban, Maria Bercea, Alina G. Rusu, Alina Ghilan, Maria Butnaru, Liliana Mititelu-Tartau, Aurica P. Chiriac, Macromol Biosci, 2023, e2200451. doi: 10.1002/mabi.202200451 (IF 5,859)
5. Alina Ghilan, **Alexandra Croitoriu**, Aurica P Chiriac, Lorena Elena Niță, Maria Bercea, Alina Gabriela Rusu, Injectable Networks Based on a Hybrid Synthetic/Natural Polymer Gel and Self-Assembling Peptides Functioning as Reinforcing Fillers, *Polymers*, 2023, 15(3):636. https://doi.org/10.3390/polym15030636 (IF 5,0)
6. Alexandra Croitoriu, Aurica P. Chiriac, Alina Gabriela Rusu, Alina Ghilan, Diana Elena Ciolacu, Iuliana Stoica, Loredana Nita, Morphological Evaluation of Supramolecular Soft Materials Obtained through Co-Assembly Processes, Gels 9(11):886. DOI: 10.3390/gels9110886 (IF 4,6)

- ***Oral communications at scientific events***

1. **Alexandra Croitoriu**, Loredana E. Nita, Alina G. Rusu, Andrei Cimponeriu, Aurica P. Chiriac, Supramolecular Gels Based on Amino Acids, *The 9th IEEE International Conference on E-Health and Bioengineering* - EHB 2021, 18-19 November, Iasi, Romania.
2. **Alexandra Croitoriu**, Alina G. Rusu, Alexandra Bargan, Maria Bercea, Liliana Mititelu-Tartau, Loredana E. Nita, Aurica P. Chiriac, Hybrid porous materials peptide-alginate/poly (itaconic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane) based with controllable properties, *3rd International Conference on Aerogels for Biomedical and Environmental Applications*, 5-7 July 2023, Maribor, Slovenia.
3. **Alexandra Croitoriu**, Loredana E. Niță, Onur Yilmaz, Development of interpenetrating network gels based on low molecular weight gelators and synthetic polymer for treating dye-contaminated water, *7th International Congress on Innovative Aspects for Leather Industry* – IAFLI 2023*,* 23-24 November, Izmir, Turcia.

- ***Poster presentations***

1. **Alexandra Croitoriu**, Loredana E. Niță, Alexandru Serban, Alina G. Rusu, New gels preparation based on peptide-peptide co-assembly, *31st Conference of the European Society for Biomaterials,*

ESB 2021 together with 43RD ANNUAL CONGRESS OF IBERIAN SOCIETY OF BIOMECHANICS AND BIOMATERIALS (SIBB), 5-9 September, 2021, PORTO, PORTUGAL.

2. **Alexandra Croitoriu**, Loredana E. Nita, Alina G. Rusu, Alina Ghilan, Florica Doroftei, Maria Bercea, Aurica P. Chiriac, HYBRID HYDROGELS BASED ON PEPTIDE, INTERNATIONAL CONFERENCE ON RHEOLOGY- *Understanding the Viscoelastic Behavior of Materials – Progress and Challenges,* 26 May, 2022, Iasi, Romania.
3. **Alexandra Croitoriu**, Alexandru M. Serban, Aurica P. Chiriac, Alina G. Rusu, Alina Ghilan, Loredana E. Nita, Hydrogels based on amino acids and gelling polymers*, Polymers 2022 Conference: New trends in Polymers Science, Health of the Planet, Health of the People*, 25-27 May, 2022, Turin, Italy.

The scientific activity related to the topic of the doctoral thesis consisted of two articles and four poster presentations:

- ***Scientific articles***

1. Alina Gabriela Rusu, Aurica P Chiriac, Loredana Elena Nita, Vera Balan, Alexandru Mihail Serban, **Alexandra Croitoriu**, Synthesis and comparative studies of glucose oxidase immobilized on Fe3O4 magnetic nanoparticles using different coupling agents, Nanomaterials, 2022, 12(14):2445. doi: 10.3390/nano12142445 (IF 5,719)
2. Ruxandra Mihailovici, **Alexandra Croitoriu**, Florin Nedeff, V. Nedeff, Lacramioara Ochiuz, Decebal Vasincu, Ovidiu Popa, Maricel Agop, Andreea Moraru, Danut Costin, Marcel Costuleanu, Liliana Verestiuc, Drug-Loaded Polymeric Particulated Systems for Ophthalmic Drugs Release, Molecules, 2022, 27(14):4512. doi: 10.3390/molecules27144512 (IF 4,927)
3. Alina Gabriela Rusu, Loredana Elena Niță, Irina Roșca, **Alexandra Croitoriu**, Alina Ghilan, Liliana Mititelu-Tarțău, Aurica Valentin Grigoraș, Bianca-Elena-Beatrice Crețu, Aurica P. Chiriac, Alginate-Based Hydrogels Enriched with Lavender Essential Oil: Evaluation of Physicochemical Properties, Antimicrobial Activity, and In Vivo Biocompatibility, Pharmaceutics, 2023, 15(11), 2608. doi: 0.3390/pharmaceutics15112608 (IF 5,4)

- ***Poster presentations***

1. **Alexandra Croitoriu**, Loredana E. NITA, Alina G. RUSU, Alina GHILAN, Florica DOROFTEI, Maria BERCEA, Aurica P. CHIRIAC, Investigation of an interpenetrated polymer system containing cellulose nanofibrils and a copolymacrolactone structure, INTERNATIONAL CONFERENCE ON RHEOLOGY- *Understanding the Viscoelastic Behavior of Materials – Progress and Challenges* 26 May, 2022, Iasi, Romania.

2. **Alexandra Croitoriu**, Alexandru Serban, Alexandra Bargan, Maria Bercea, Florica Doroftei, Loredana Elena Nita, Alina Gabriela Rusu, Alina Ghilan, Aurica P. Chiriac, Structured Aerogels as Advanced Materials Based on Cellulose Nanofibrils and a Copolymacrolactone System, *2nd International Conference on Aerogels for Biomedical and Environmental Applications* – AERoGELS 2022, 29 June- 01 July, 2022, Athens, Greece.
3. Loredana E. Nita, Aurica P. Chiriac, **Alexandra Croitoriu**, Alina G. Rusu, Alina Ghilan, Alexandru M. Serban, Irina Rosca, Alexandra Bargan, New antibacterial aerogels based on synthetic polymers and CNF, *2nd International Conference on Aerogels for Biomedical and Environmental Applications* – AERoGELS 2022, 29 June- 01 July, 2022, Athens, Greece.
4. Alina Gabriela Rusu, Aurica P. Chiriac, Loredana Elena Nita, Alina Ghilan, **Alexandra Croitoriu**, Bianca Elena Beatrice Cretu, Alexandru Mihail Serban, Alexandra Bargan, Florica Doroftei, Design of agarose based aerogels with potential application as wound dressings, *3rd International Conference on Aerogels for Biomedical and Environmental Applications, 5-7 July* 2023, Maribor, Slovenia.

- ***Patents***

1. Loredana Elena Niță, Aurica Chiriac, Alina Gabriela Rusu, **Alexandra Croitoriu**, Alexandru Șerban, Iordana Neamțu, Constanța Munteanu, Procedeu de preparare a unui gel autoasamblat pe bază de peptide, RO 137294-A8.

- ***Awards at scientific events***

1. Young Researcher, 9th IEEE International Conference on E-Health and Bioengineering - EHB 2021.

- ***Research contracts***

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1. ”New hybrid polymer/peptide hydrogels as innovative platforms designed for cell cultures applications” - PN-III-P2-2.1-PED-2019-2743.
2. ”3D Advanced smart magnetic scaffolds for bone tissue engineering and regeneration” - PN-III-P2-2.1-PED-2019-4524.
3. ”Hybrid bio-systems enriched with biotechnological extracted oils and applicability in skin tissue engineering” - PN-III-P2-2.1-PED-2021-2229.
4. 3D bio-inspired hybrid architectures for deep thickness skin repair and regeneration - PN-III-P2-2.1-PED-2021-3003.

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