

**Intermediate synthetic scientific report**  
*On the implementation of the project*  
**NEW POLYMER / PEPTIDE HYBRID HYDROGELS AS**  
**INNOVATIVE PLATFORMS**  
**DESIGNED FOR CELL CULTURE APPLICATIONS**

**STAGE II / 2021**

**Study of the phenomenon of self-assembly in solution of peptides as low molar weight crosslinkers (LMMGs) - continuation of studies started in 2020**

**Formulation of the concept on the preparation of hybrid materials based on LMMG / PG (gelling polymers)**

**Formulation of the concept of preparation of multicomponent gels**

**A1: Study of the ability of peptides co-assembly - continuation of studies started in 2020.**

**A2: Study of the effect of specific factors on the ability of peptides to form supramolecular structures - continuation of studies started in 2020**

**A3: Selection of supramolecular assemblies based on LMMGs synthesized in Stage I by correlating the results obtained from the physico-chemical properties**

**A4: Preparation of hybrid materials**

**A5: Synthesis of double networks (DN) using a preformed gel**

**A6: Synthesis of DN by forming "in situ" of the second network**

**A7: Characterization of the obtained gels**

**A8: Dissemination**

**Results delivered on stage:**

(1) self-assembled peptide-based structures such as LMMGs ;

(2) hybrid gels based on natural polymers and LMMG

(3) DN structures based on a preformed gel; DN structures, in which a network is obtained in situ; multicomponent gels

The team involved in the scientific development of Stage II / 2021

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## • Stage Summary

Supramolecular chemistry includes and investigates highly ordered systems formed by intra- and intermolecular combinations of weak and reversible non-covalent interactions.

Low molecular weight crosslinkers (LMMG) are a class of compounds (size <3kDa) that have the ability to self-associate through physical interactions (hydrogen bonds, ionic, hydrophobic, van der Waals,  $\pi$ - $\pi$  conjugation) in a three-dimensional network and have the ability to incorporate solvent molecules into their structure.

Amino acids and peptides, as short chain amino acids coupled by peptide bonds, are organic structures that can be included in the category of low molecular weight crosslinkers. Peptide-based supramolecular gels are of particular importance in biomedical applications due to their remarkable intrinsic properties.

This class of soft materials is used, in particular, in the administration of drugs, wound dressing due to its antibacterial action and repairing effect, or as a 3D support for the development of cell cultures. Peptides are considered attractive for biomedical fields and due to the mimetic biochemical processes of natural extracellular matrices (ECM), nanofiber aspects and high hydration capacity. The main advantages of these small molecules are biocompatibility, bioactivity, adhesion and cell proliferation. These characteristics are essential for the development of three-dimensional structures with a role in cell growth.

The main objective of the project is to synthesize new hybrid polymer / peptide hydrogels as innovative platforms designed for applications in cell cultures. In this context, the aim was to diversify the already existing range by introducing new structures that would bring benefits such as better resistance, more structured networks. Starting from these aspects and in accordance with the work plan, in this stage were studied:

- **Ability to co-assemble peptides** (continuation of studies started in 2020): **The study of the co-assembly capacity highlighted the following aspects regarding the chosen systems:** (i) some systems gelled under any of the chosen conditions (Fmoc-Lys-Fmoc + Fmoc-Phe-Phe system), (ii) some systems did not gel regardless of the chosen working conditions (Fmoc-Gly-Gly-Gly + Fmoc-Ga, Fmoc-Gly-Gly-Gly + Fmoc-Phe-Gly) and (iii) there are systems that gelled only under certain conditions (Fmoc-Lys-Fmoc + Fmoc-Serine, Fmoc-Lys-Fmoc + Fmoc-Ga, Fmoc-Lys-Fmoc + Fmoc-Gly-Gly-Gly, Fmoc-Phe-Phe + Fmoc-Gly-Gly-Gly) but which formed stable and transparent gels.

- **Study of the effect of specific factors on the ability of peptides to form supramolecular structures** (continuation of studies started in 2020): The influence of pH, solvent and concentration on the self-assembly process in peptide systems was studied. The gelling process was found to be influenced by both changes in pH and the use of a polar solvent (DMSO).

- **Selection of supramolecular assemblies based on LMMGs by correlating the results obtained from the physico-chemical properties:** Following the results obtained from the processes of preparation of supramolecular gels using specific peptide structures The following systems were chosen for evaluation and characterization: Fmoc-Lys-Fmoc + Fmoc-Gly-Gly-Gly; Fmoc-Lys-Fmoc + Fmoc-Serine; Fmoc-Lys-Fmoc + Fmoc-glutamic acid. The samples were characterized using spectroscopic techniques (FTIR, DC, fluorescence, UV-VIS), rheological studies, SEM morphology. It turned out that the system based on Fmoc-Lys-Fmoc + Fmoc-Gly-Gly-Gly best meets the characteristics of a supramolecular gel.

- **Preparation of hybrid materials:** By combining LMMG with a polymer with gelling capacity (agarose, phytigel) hybrid gels with a stable structure were obtained as shown by the characteristics (FTIR spectra, overthrow of the vial, thermal analysis, SEM morphology ) undertaken. The gels prepared after the co-assembly process have a transparent appearance. The samples in the ratio of 3/1 Fmoc-Lys-Fmoc / natural polymer showed a better viscoelastic behavior and a translucent appearance, while the samples with low content of Fmoc-Lys-Fmoc are less stable and more opaque.

**- Synthesis of double networks (DN) using a preformed gel:** Poly (itaconic anhydride-co-3,9-divinyl-2,4, 8, 10-tetraoxaspiro (5,5) undecane) (PITAU), a synthetic copolymer with functional groups, synthesized within the team and having a patented structure, was used. PITAU has specific properties, such as the ability to create networks, biodegradability and biocompatibility, binding properties, amphiphilicity, thermal stability, and sensitivity to pH and temperature. The possibility of forming 3D networks based on PITAU and alginate was investigated, in order to obtain biocompatible gels with improved properties. The obtained structures were characterized (FTIR, thermal analysis, SEM) and the formation of the network was confirmed.

**- Synthesis of DN by the formation "in situ" of a synthetic polymeric network** (poly (dimethylaminoethyl methacrylate) (PDMAEMA)) in the interpenetrating meshes of the supramolecular network formed by amino acid / peptide. The obtained structures were characterized (FTIR, thermal analysis, SEM) and the formation of the network was confirmed.

## **Dissemination**

### **Articles:**

1. Alginate enriched with phytic acid for hydrogels preparation; L. E. Nita, A. P. Chiriac, A. Ghilan, A.G. Rusu, N. Tudorachi, D. Timpu; International Journal of Biological Macromolecules 181, 561 (2021). **IF=6.953**
2. Alginate enriched with phytic acid for hydrogels preparation. Therapeutic applications, LE Nita, AP Chiriac, A Ghilan, AG Rusu, D Pamfil, I Rosca, L Mititelu-Tartau, International Journal of Biological Macromolecules, 189, 335 (2021). **IF=6.953**
3. Co-assembled peptides hierarchically oriented for supramolecular gel formation; A. Croitoriu, L.E. Nita, A. G. Rusu, F. Doroftei, L. Verestiuc Rev. Roum. Chim., 65(5), 449 (2021). **IF=0.418**
4. New physical hydrogels based on post-assembly of Fmoc-amino acids, A. Croitoriu, L. E. Nita, A. P. Chiriac, A. G. Rusu, M. Bercea, Gels 7, 208 (2021). **IF=4.702**
5. New hydrogel network based on alginate and a spiroaceta copolymer, A. E. Sandu, L. E. Nita, A. P. Chiriac, N Tudorachi, A. G. Rusu, D Pamfil, Gels, 7, 241 (2021). **IF=4.702**
6. Synthesis of poly(Ethylene brassylate-co-squaric acid) as potential essential oil Carrier, A. P. Chiriac, A. G. Rusu, L. E. Nita, A.-M. Macsim, N. Tudorachi, I. Rosca, I. Stoica, D. Timpu, M. Aflori, F. Doroftei; Pharmaceutics, 13, 477 (2021). **IF=6.07**
7. Polymeric carriers designed for encapsulation of essential oils with biological activity, A. P. Chiriac, A. G. Rusu, L. E. Nita, V. M. Chiriac, I. Neamtu, A. Sandu; Pharmaceutics 13, 631 (2021). **IF=6.07**

**Patent request: A/00465/05.08.2021:** PROCEDEU DE PREPARARE A UNUI GEL AUTOASAMBLAT PE BAZA DE PEPTIDE, Autori : LE Nita, AP Chiriac, AG Rusu, A Croitoru, MA Serban, I Neamtu, C Munteanu.

### **Paper published in ISI Conference Proceeding:**

1. A.Raicu, I.Cobzariu, A.L.Vasilache, C.A. Peptu, M. Butnaru, L.Verestiuc, New Hydrogels Based on Methacrylated Collagen and Hyaluronic Acid for Soft Tissue Engineering (Invited), 5th INTERNATIONAL CONFERENCE on Nanotechnologies and Biomedical Engineering, November 3-5, 2021, Chisinau, Republic of Moldova
2. A. Luca, T.R. Craescu, L. Verestiuc, M. Butnaru, Mimicking In Vivo Tissue Microenvironment for In Vitro Testing –Hydrogels for Cell Encapsulation, 5th INTERNATIONAL CONFERENCE on Nanotechnologies and Biomedical Engineering, November 3-5, 2021, Chisinau, Republic of Moldova
3. Roxana Donea, Isabella Cobzariu, Maria Butnaru and Liliana Verestiuc, , IEEE E-HEALTH AND BIOENGINEERING CONFERENCE - EHB 2021, 9-th edition, Iasi, Romania, November 18-19, 2021
4. Supramolecular gels based on amino acids, A. Croitoriu, L. E. Niță, A. G. Rusu, A. Cimponeriu, A. Chiriac, IEEE International Conference on e-Health and Bioengineering EHB 2021 - 9-th Edition, 18-19 November 2021, Iasi - WEB CONFERENCE – Romania

5. New polymeric particles loaded with sea buckthorn essential oil, A. E. Sandu, A. Chiriac, A. G.Rusu, A. M. Serban, L. E. Niță, E. Gille, IEEE International Conference on e-Health and Bioengineering EHB 2021 - 9-th Edition, 18-19 November 2021, Iasi - WEB CONFERENCE - Romania

**Paper published in Conference Proceeding:**

Isabella Cobzariu, Maria Bercea, Catalina Anisoara Peptu, Maria Butnaru, Liliana Verestiuc, 3D Bioprinted scaffolds based on functionalised gelatin and PEGDA for soft tissue engineering, Proceedings International Conference Progress in Organic and Macromolecular Compounds, International Conference Progress in Organic and Macromolecular Compounds, 28th Edition, Iasi | Romania | October 7 - 9, 2021, ISSN 2810 – 2347, pp 47-48

**Oral presentations**

1. Supramolecular gels based on amino acids, A. Croitoriu, L. E. Niță, A. G. Rusu, A. Cimponeriu, A. Chiriac, IEEE International Conference on e-Health and Bioengineering EHB 2021 - 9-th Edition, 18-19 November 2021, Iasi - WEB CONFERENCE – Romania
2. New polymeric particles loaded with sea buckthorn essential oil, A. E. Sandu, A. Chiriac, A. G.Rusu, A. M. Serban, L. E. Niță, E. Gille, IEEE International Conference on e-Health and Bioengineering EHB 2021 - 9-th Edition, 18-19 November 2021, Iasi - WEB CONFERENCE - Romania

**Poster presentations**

New gels preparation based on peptide-peptide co-assembly, Alexandra Croitoriu, Loredan Elena Nita, Alexandru Serban, Alina Gabriela Rusu, 31<sup>st</sup> Annual Conference of the European Society for Biomaterials (ESB 2021), online, 5-9 Septembrie 2021