PERIODICO di MINERALOGIA Volume 89, No. 3, 2020

Preparation of an Elastin–Collagen Artificial Matrix. Evaluation of Its Structure and Biocompatibility

OANA CRACIUNESCU[*], LUCIA MOLDOVAN[*], WANDA BUZGARIU[*], OTILIA ZARNESCU[**], D. BOJIN[***], G.L. RADU[*]

[*]*National Institute R-D for Biological Sciences, 296, Spl. Independentei, 060031, P.O. Box 17-16, Bucharest, Romania*

[**]*Faculty of Biology, University of Bucharest*

[***]*Faculty of Metallurgy, University "Polytechnic" Bucharest*

Abstract

The biochemistry of the reaction between soluble elastin and collagen was analyzed. The best association parameters were settled. The elaboration of two variants of artificial matrix based on soluble elastin (type α or k) and collagen was described. Structurally, the membrane α-EL:COL, in 1:20 ratio, was homogeneous and stable. The viability of the cells cultivated in the presence of this variant and of its constituents was higher than 95%. In conclusion, among the composite bioproducts, α-EL:COL 1:20 was selected as the best for burn skin treatment.

Keywords: soluble elastin, collagen, bioproduct, microscopy, biocompatibility

Introduction

Most biomaterials developed for skin lesion treatment are made of collagen (COL) or mixtures of COL and glycosaminoglycans (GAG). The use of elastin (EL) in combination to COL is justified by several reasons. Immunological studies revealed that elastin matrix induced a lower density of activated leukocytes than COL. This reduced trombogenicity is in favour of elastin use in tissue implants as blood contacting material [1]. Also, EL is a constitutive protein of the matrix and contributes to the morphogenesis [2] and control of cellular activity [3]. Elastic peptides stimulate elastic fibres adhesion to fibroblasts [4] and can be adsorbed and desorbed, in particular conditions, on natural and synthetic polymers [5].

Until now, EL was seldom used in bioproducts' composition. A matrix of EL with fibrin was obtained in the presence of COL type I [6]. It were prepared gels and scaffolds from COL and insoluble EL [7, 8, 9]. Other teams used the EL from isolated blood vessels [10, 11] or heart valves [12]. Also, EL hydrolysates were used in biomaterial elaboration [13, 14].

The aims of this study were the soluble EL-COL interaction analysis, the elaboration of a bioproduct and the evaluation of its cytocompatibility and structure, in order to select the best variant for burnt or mechanical harmed skin treatment.

Materials and Methods

Materials. α-EL solution was obtained from insoluble EL treated with 0.25M oxalic acid, at 100^0C [15]. k-EL solution was obtained by alkaline treatment, at 37^0C, of the insoluble EL [16]. Solutions of COL type I from rat tail tendon and COL types I+III from bovine hides were prepared, as described [17].

Methods.

Turbidimetric method. The reaction between a 0.5% hydrolyzed EL solution and 0.5% COL solution was turbidimetrically analyzed. The variation in time, depending on the temperature, pH, ionic strength, and concentration was registered at 405nm, with a CECIL 1020 spectrophotometer.

Elaboration and conditioning of the bioproduct. 2 variants of bioproduct were obtained by slowly pouring of a 0.5% COL types I+III solution, over a 0.5% soluble EL solution. The mixtures with different ratios EL:COL (1:20 or 1:10) were incubated 30 min., at 37^0C, put in a mould and dried, resulting in flexible membranes.

Microscopy analysis. For light microscopy, membrane samples were fixed in Bouin solution and embedded in paraffin. 7 μm sections were stained with orcein for elastic fibres. For electron microscopy (SEM), membrane samples were processed in the 'low vacuum' mode and visualized at a scanning electron microscope ESEM, XL-30, FEI (Philips).

In vitro cell viability test. The α-EL and k-EL solutions, obtained from insoluble EL by acid and alkaline hydrolysis, respectively, together with the EL-COL membranes were tested on a primary culture of embryonic fibroblasts, at third passage, in order to analyze a possible release of toxic products. For this, the cells were cultivated in the presence of EL peptides or were seeded on the membranes and cultivated in cell culture medium, DMEM supplemented with 10% fetal calf serum and antibiotics, for 72 h, at 37^0C (5% CO_2). Cell viability was quantitatively determined in the samples after 24h and 72 h, by MTT test [18]. The cell viability was expressed as percent considering the control value as 100%.

Results and Discussions

1. *The biochemistry of soluble EL – COL interaction*

1.1. *The kinetics of the reaction* was studied spectrophotometrically. The variation in time of the turbidity for the soluble EL solution and the soluble EL-COL mixtures, in 1:10 and 1:20 ratios, is presented in **Figure 1**.

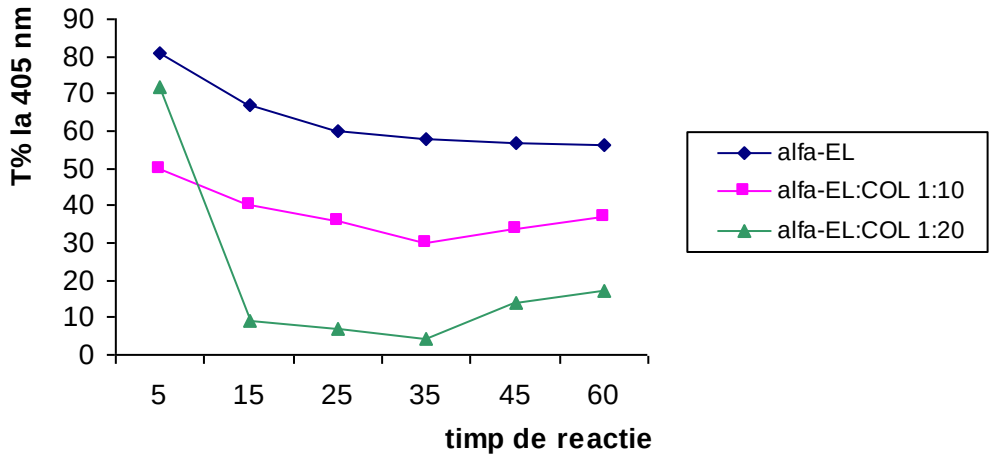

Figure 1 – Kinetics curve of α-EL-COL I+III reaction

The turbidity of the EL-COL mixture, in 1:10 ratio, decreased, confirming the aggregation between the proteic components. A rapid diminution of the turbidity value to 4.5% was observed when a larger quantity of COL was present in the reaction mixture (1:20 ratio).

Thus, the combination ratio of 1:20 EL:COL, similar to their proportion in skin extracellular matrix, seems to be appropriate for a stable form of the bioproduct.

1.2. *Collagen type influence*. When a soluble EL solution was mixed with a COL type I+III solution, a variation of approx. 40% in the turbidity value was observed after 30 min. (**Figure 2**). Using COL type I, the registered variation curve had a slower slope (~15%).

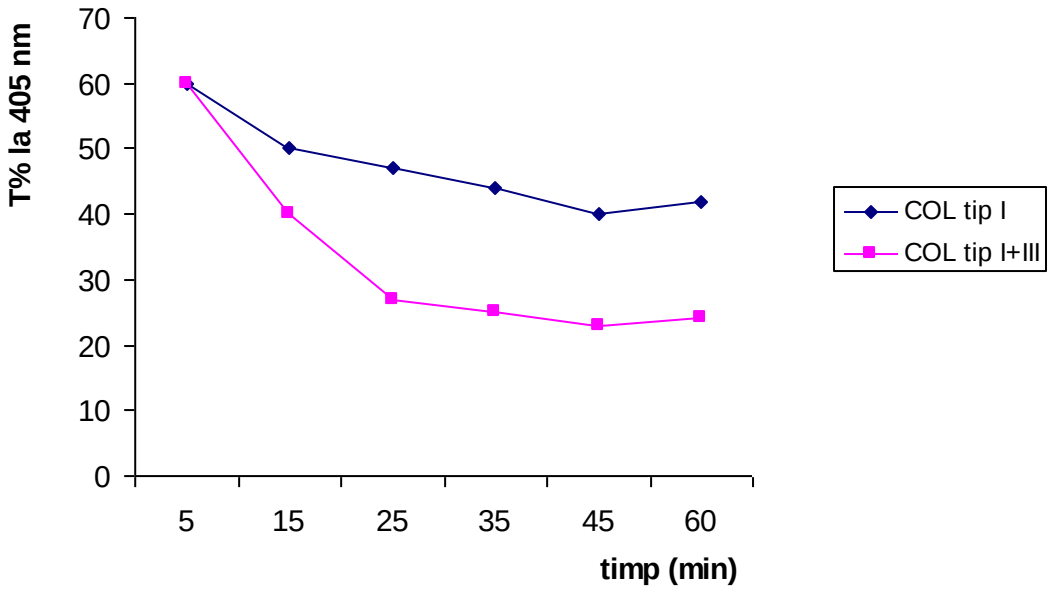

Figure 2 – Collagen type influence on the reaction kinetics

Previous studies used pure COL type III and demonstrated that a greater quantity of EL interacted [12]. These results showed the important role of type III COL in EL aggregation. The observation that, in fetal skin, type III COL is present in a higher percentage than in adult skin and, also, in wounds as compared with normal tissues, is an argument for its use in the bioproduct's composition [7].

1.3. *Temperature influence*. The temperature played an important role in soluble EL-COL interaction. The results showed that, at 37^0C, the turbidity was immediately lowered after adding the COL solution, in comparison to the reaction proceeded at room temperature (**Figure 3**).

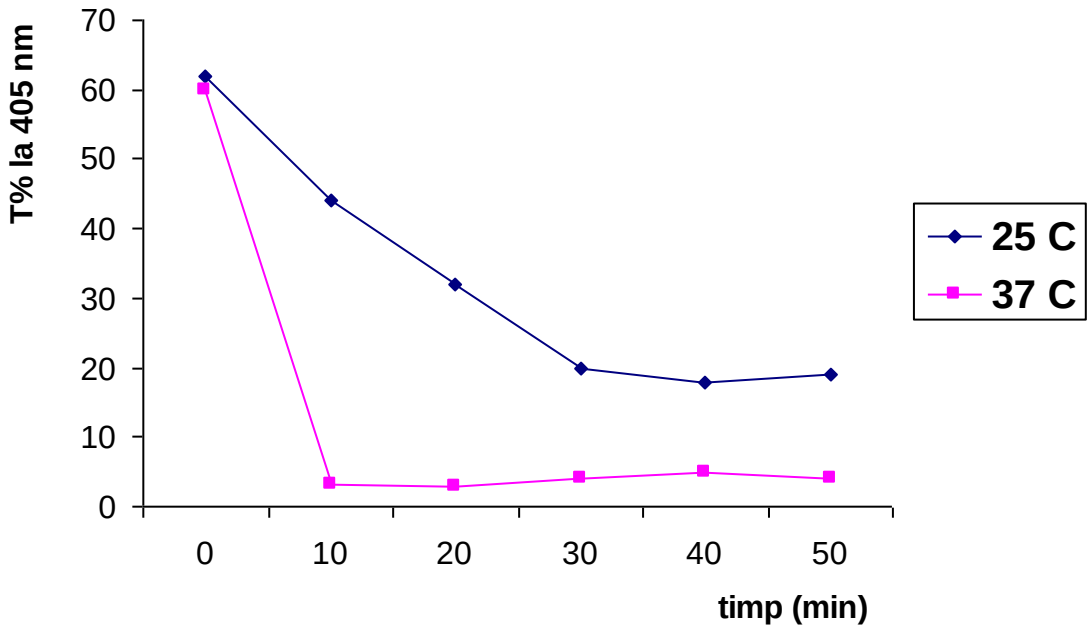

Figure 3 – Temperature influence on EL-COL reaction

1.4. *pH and ionic strength influence*. The tested pH range was 3.5 to 9. The acid or alkaline environment hindered the EL-COL interaction. In turn, at pH 7.4, the component aggregation took place in short time after the mixing (T%=20).

The presence of calcium and zinc salts in the reaction mixture didn't modified the biochemistry, but the resulting membranes lost their transparency.

From the biochemical results it is concluded that soluble EL-COL *in vitro* interaction must be carried out at the physiological values (temperature of 37^0C, pH 7.4, combination ratio EL:COL 1 :20), as the best and reproducible conditions, in order to obtain a medical bioproduct. Using physiological conditions, the EL maintained its native physical and chemical properties (elasticity, mechanical strength, impermeability) and its structure retained a specific polarity and the capacity to interact electrostatically.

2. Structural analysis of the obtained artificial matrices

Mixing the main purified components of the extracellular matrix (ECM), in different ratios, we can obtain a diversity of bioproducts with various biochemical, biomechanical and morphological characteristics [5]. Thus, it can be controlled the composition (by choosing the ECM constituents and their ratio), the mechanical properties and the pore size of the bioproduct.

Light microscopy studies revealed that, EL fibers were spread on the surface of the membrane EL:COL, in 1:20 ratio (**Figure 4**). Also, COL acted like a binding material and defining a stable bioproduct. In turn, the EL-COL membrane, in 1:10 ratio, was more fragile, with a lax structure, probably due to the EL fibres present as large groups (**Figure 5**).

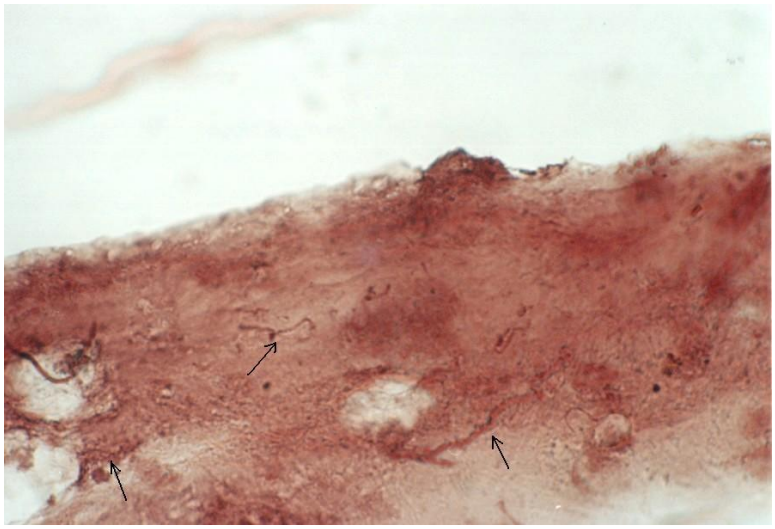

Figure 4 – Light micrograph of the EL:COL 1:20 membrane. There are elastin fibres on the membrane's surface (arrows) (orcein staining, x400).

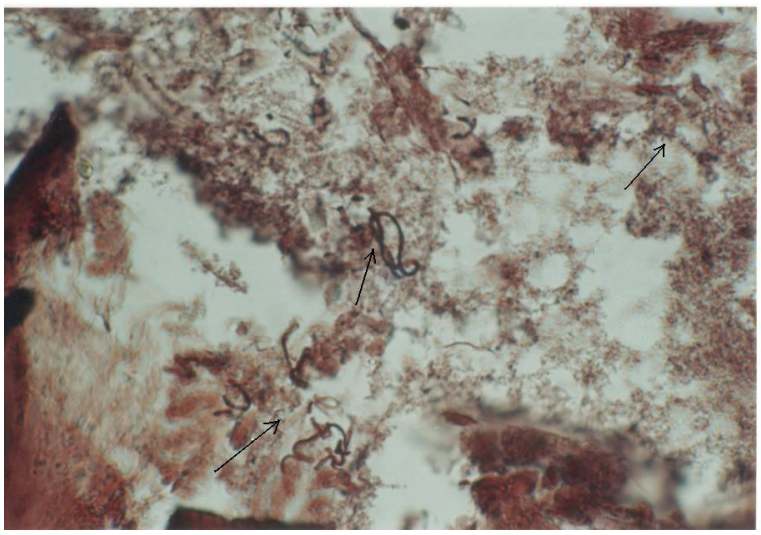

Figure 5 – Light micrograph of the EL:COL 1:10 membrane. It were observed elastin fibres groups (arrows) (orcein staining, x400).

By SEM, all the analyzed variants presented a three-dimensional, fibrillar and dense structure. The presence of EL induced the formation of a macromolecular aggregate with a

different structure from that of the individual components. There was a uniform surface and the fibers were mostly orientated parallel or perpendicular to the surface, regardless of the elastin type (**Figure 6**).

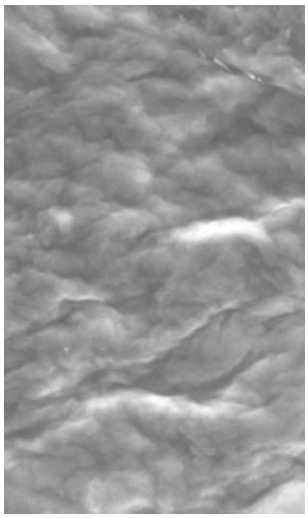

Figure 6 – Scanning electron micrograph of a cross section in EL:COL membrane (x2500).

Although the bioproduct's microstructure was dense and didn't allow cell infiltration, the membrane might play some biological roles of the skin : acting like a bacterial barrier, allowing the oxygen passing and maintaining the humidity [19].

3. Cytocompatibility evaluation

MTT test results revealed a high cellular viability for the matrix components, α-EL, k-EL, COL (**Figure 7**).

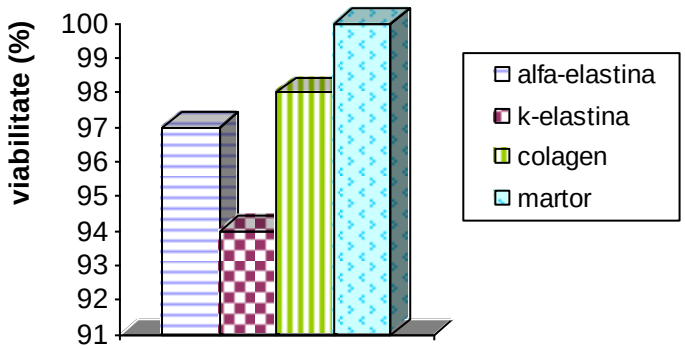

Figure 7 – Diagram of embryonic fibroblast viability in the presence of individual matrix components

The cell viability values for the membranes, after 24h and 72h, diminished as follows: α-EL:COL 1:20, α-EL:COL 1:10, k-EL:COL 1:20 (**Figure 8**).

Figure 8 – Diagram of embryonic fibroblast viability in the presence of EL-COL membranes, determined by the MTT method.

These results were favourable to α-EL membranes, due to a viability value greater than 90%.

Conclusions

The best conditions for soluble EL-COL interaction were: a temperature of 37^0C, pH 7.4 and a EL:COL ratio of 1:20. The type of EL didn't influenced the reaction, but the use of COL types I+III was necessary, in order to obtain a stable macromolecular aggregate.

Structurally, the EL:COL membrane, in 1:20 ratio, was homogeneous, comprising EL fibres uniformly spread. The EL:COL membrane, in 1:10 ratio, presented a lax structure and EL fibres groups. By SEM, the surface of the membrane was uniform and the fibers were mostly parallel and perpendicular orientated.

The cell viability in the presence of the α-EL:COL membrane variant, in 1:20 ratio, was higher than 95%.

Acknowledgement

This research was supported by Project No. 179 VIASAN.

References

1. S.Q. LIU, C. TIECHE, P.K. ALKEMA, *Biomaterials*, **25**, 101-115 (2004).
2. D.Y. LI, B. BROOKE, E.C. DAVIS, R.P. MECHAM, L.K. SORENSEN, B.B. BOAK, E. EICHWALD, M.T. KEATING, *Nature*, **393**, 276-280 (1998).
3. A. HINEK, *Biol. Chem*., **377**, 471-480 (1996).
4. V. GROULT, W. HORNEBECK, P. FERRARI, J.M. TIXIER, L. ROBERT, M.P. JACOB, *Cell Biochem. Funct.*, **9**, 171-182 (1991).
5. S. DUTOYA, F. LEFEBVRE, C. DEMINIERES, F. ROUAIS, A. VERNA, A. KOZLUCA, A.M. LE BUGLE, *Biomaterials*, **19**(1-3), 147-155 (1998).
6. M.APRAHAMIAN, A. LAMBERT, G. BALBONI, F. LEFEBVRE, R. SCHMITTHAEUSLER, C. DAMGE, M. RABAUD, *J. Biomed. Mater. Res*., **21**, 965-977 (1987).

7. M. RABAUD, F. LEFEBVRE, D. DUCASSOU, *Biomaterials*, **12**, 313-319 (1991).
8. D.W. COURTMAN, B.F. ERRETT, G.J. WILSON, *J. Biomed. Mater. Res*., **55**, 576-586 (2001).
9. W.F. DAAMEN, H.Th.B. van MOERKERK, T. HAFMANS, L. BUTTAFOCO, A.A. POOT, J.H. VEERKAMP, T.H. van KUPPEVELT, Biomaterials, **24**, 4001-4009 (2003).
10. F.J. SCHOEN, R.J. LEVY, *J. Biomed. Mater. Res*., **47**, 439-465 (1999).
11. Q. LU, K. GANESAN, D.T. SIMIONESCU, N.R. VYAVAHARE, *Biomaterials*, **25**, 5227-5237 (2004).
12. F. LEFEBVRE, S. GORECKI, R. BAREILLE, J. AMEDEE, L. BORDENAVE, M. RABAUD, *Biomaterials*, **13**, 28-33 (1992).
13. N. BONZON, X. CARRAT, C. DEMINIERE, G. DACULSI, F. LEFEBVRE, M. RABAUD, *Biomaterials*, **16**, 881-885 (1995).
14. A. SINGLA, C.H. LEE, *J. Biomed. Mater. Res*., **60**, 368-374 (2002).
15. J.M. DAVIDSON, M.G. GIRO, R.P. MECHAM, *Extracellular Matrix*, M.A. Haralson, J.R. Hassell, eds., IRL Press, Oxford, 1995, pp. 241-260.
16. S. DUTOYA, A. VERNA, F. LEFEBVRE, M. RABAUD, *Biomaterials*, **21**, 1521-1529 (2000).
17. G. NEGROIU, N. MIRANCEA, D. MIRANCEA, A. OANCEA, L. MOLDOVAN**,** *Rev. Roum. Biochim.*, **29**(1), 23-28 (1992).
18. F. DENIZOT, R. LANG, *J. Immunol. Meth.*, **89**, 271-277 (1986).
19. L. MA, C. GAO, Z. MAO, J. ZHOU, J. SHEN, X. HU, C. HAN, *Biomaterials*, **24**, 4833-4841 (2003).