Collagenic membranes modified with natural compound for improved bio-integration: structural, morphological and histological analysis

S. Cavalu[a], F. Beteg[b], P. M. Pasca[a], M. Moisa[a], O. Pop[a], A. Antonescu[a], C. Cheregi[a,*], F. Manole[a]
[a]*Faculty of Medicine and Pharmacy, University of Oradea, P-ta 1 Decembrie 10, 410087, Oradea, Romania*
[b]*Department of Clinical Sciences, University of Agricultural Sciences and Veterinary Medicine, 400374 Cluj-Napoca, Romania*

Collagen membranes have been widely investigated in animal studies and human clinical studies, and have demonstrated excellent biocompatibility, biodegradability and cell affinity. Membrane porosity and 3D architecture are considered to be crucial for cellular infiltration and proliferation, in the process of wound heling. In this context, the aim of our study was the prepare and to investigate comparatively the structural and morphological properties of collagenic membranes modified with a natural bio-compound (respectively azelaic acid) and to evaluate their bio-integration and immune response in the framework of an animal model. Our results shown a porous structure with a honeycomb-style architecture achieved as a result of azelaic acid incorporation in collagenic membrane, with a beneficial effect on tissue remodelling and rapid healing. The bio-integration of azelaic acid-collagenic membrane was faster compared to pure collagenic one, with only minor inflammatory events.

(Received April 4, 2023; Accepted July 4, 2023)

Keywords: Collagenic membrane, Azelaic acid, Wound healing, Bio-integration.

1. Introduction

Collagenic membranes from natural sources are extensively used in clinical practice as skin substitute, wound healing dressing, guided bone regeneration or vascular patches, due to their excellent biocompatibility, biodegradability and cell affinity [1-3]. Currently, many manufacturers and companies are developing a variety of collagen-based films or membranes derived from human or animal sources with different structures and crosslinking technology [3]. A special attention was paid to collagenic materials for wound healing, in the context of the complex healing process which depends on multiple factors such as the presence of multiple types cells, regulation processes involved in haemostasis, inflammation, proliferation and tissue remodelling. The interaction between extra cellular matrix (ECM) proteins and different cells triggers the inflammatory response and phagocytosis stimulated by foreign bodies or damaged tissue present in the wound site. It is well known that platelets activation, cytokines secretion, macrophages, fibroblasts and keratinocytes are the most important active factors to promote wound closure and formation of new tissue [4-6].

Depending on the purpose, the biochemical pathway resorption over time has to be taken into account, as the main disadvantage of natural collagenic membranes is their unpredictable degree of resorption, with possible consequences in terms of bio-integration, immune response and successful cutaneous tissue healing [7-9]. From this point of view, the clinical performance of currently available collagen membranes seems to be unsatisfactory, and the continuous need to improve their clinical performance emerged as an important trend in tissue regeneration applications.

Usually, in order to improve the mechanical properties of biogenic collagen membranes, cross-linking procedures are performed either by physical, enzymatic or chemical approach [8]. The main drawbacks of using chemical cross-linking (which is the most effective method and low cost),

* Corresponding author: cornel.cheregi@yahoo.com
https://doi.org/10.15251/DJNB.2023.183.783

involves the potential cytotoxicity and inflammatory response in the surrounding tissue [10]. Other approach is based on modification of the collagenic membranes by using natural reagents such as polyphenols [11], honey [12], propolis [13], plasma rich in growth factors (PRGF) [2] or doping the collagen matrix with nutritionally fundamental trace elements such as zinc, magnesium, or strontium ions [14].

According to recent literature, collagen matrices and scaffolds were developed for tissue engineering, soft tissue repair and as a drug delivery system incorporating additives such as insulin [15], antibiotics [16] or nanoparticles [17] and have been tested in small animal models of wound healing. However, a lack of high-quality studies and randomized control trials can be noticed.

Multifunctional, natural molecules are good alternatives for antibiotics used in wound healing, as the local infection after skin injury is an important factor affecting wound healing process. Azelaic acid, as a natural product, was proven to be effective in targeting multiple dermatological conditions, by exerting a bacteriostatic effect on both aerobic and anaerobic bacteria, but also producing a direct anti-inflammatory effect due to its ability to neutralize free oxygen radicals [18]. Azelaic acid is a dicarboxylic acid that occurs naturally, but also industrially produced by the ozonolysis of oleic acid. Naturally, it is produced by the fungus *Malassezia furfur* (a yeast that lives on normal skin), but also found in whole grain cereals, rye, barley, and even animal products [19]. Saturated dicarboxylic acids possess the ability to inhibit membrane-associated thioredoxin reductase reversibly, based on two mechanisms: 1) by directly reducing free radicals and 2) regulating melanin biosynthesis within NADPH/TR/thioredoxin/tyrosinase pathway. Hence, azelaic acid is a rational choice for the treatment of mild-to-moderate cases of skin disorder, owing the anti-inflammatory, antioxidative effects and is bactericidal action against a range of Gram-negative and Gram-positive microorganisms, including antibiotic-resistant bacterial strains [20].

In the light of recent developments in the modern management of wound healing, the aim of our work was to prepare and to evaluate from the structural and morphological point of view a novel collagenic membrane modified with azelaic acid. We also aimed to evaluate the bio-integration capacity and the immune response generated by the tissue in the framework of a small animal model.

2. Materials and methods

2.1. Preparation of collagen/azelaic acid membrane

Azelaic acid (powder, min 98%) was provided by company MYAM Elemental SRL, Oradea, Romania. Collagen from calf skin was purchased from Sigma-Aldrich and dissolved in 0.1 M acetic acid solution to obtain a protein concentration of 2 % (w/v). Collagen solution was completely solubilized by heating at 70^{o}C for 15 min, concomitant with magnetic stirring at 400 rpm. The obtained uniform solution was divided in two equal parts in order to prepare pure respectively modified collagen membranes. The modified collagen solution was prepared by adding homogeneous mixture of phosphate buffer pH = 7.6 and azelaic acid solution of concentration 25% in ratio 10:1 (v/v), while continuous stirring, until homogenous mixture was obtained. Both collagen solutions (with and without azelaic acid) were cast onto a glass plate while the solvent was volatized using compressed air jets, at room temperature, and then kept for 6 hours in a sandwich system, between two glass plates, manually pressed. Then, the resulted films were carefully detached and submitted to further analysis.

2.2. Structural and morphological characterization

Both membranes (with and without azelaic acid incorporated) were investigated by FTIR (Fourier Transform Infrared Spectroscopy) in the range 400-4000 cm^{-1}, using Spectrum BXII spectrophotometer (Perkin Elmer), equipped with ATR accessory (ZnSe crystal), at scanning speed of 32 cm^{-1} and spectral width 2.0 cm^{-1}. The structural details of the powder azelaic acid (as received from the supplier) were also investigated by FTIR in order to allow spectral comparison. The morphological details of the films were observed by SEM (JEOL JSM7000 F), and the images were recorded both on the surface and cross-sectioned area.

2.3. Nanoindentation measurements

Both collagenic membranes were subjected to mechanical tests using the Nanoindenter G200 device (Agilent Technologies, Santa Clara, CA, USA), while the tip used to determine the mechanical properties was a diamond Berkovich, pyramidal shaped tip. The membranes were tested at room temperature and normal humidity (45–52%); 30 indentations were applied in different sites, randomly chosen on the surface of each sample, reaching a maximum of 2000 nm/s depth, while recording the forces for the load curve. The values of Young modulus were obtained from the apparatus software and load–displacement curves, by fitting parameters, using Oliver–Pharr method, as described in previous papers [2, 6].

2.4. In vivo experimental design. Development of an animal model to evaluate the bio-integration properties and immune response

In vivo study was performed under the regulations of Declaration of Helsinki and EUs Directive 2010/63/EU on the protection of animals used for scientific purposes. The protocol was approved by the Ethics Committee of Faculty of Medicine and Pharmacy, University of Oradea, Romania by decision nr. CEFMF/01/ 11.05.2022. The animals were accommodated in standard laboratory conditions and were provided with food and water *ad libitum.* A total of 27 Wistar rats, males, with average weight of 250 g were selected for this study and divided in 4 groups as following:

- Group 1 (n=9) animals allocated for subcutaneous implantation of pure collagenic membrane size 10 mm x10 mm (COL)
- Group 2 (n= 9) animals allocated for subcutaneous implantation of azelaic acid modified collagenic membrane size 10 mm x10 mm (AAC)
- Group 3 (n=9) control group underwent the surgical procedure without biomaterial insertion, the surgical cut was left to heal naturally (NAT).

Intraperitoneal anaesthesia was applied by administration of a mixture of ketamine (90 mg/kg) and xylazine (25 mg/kg) and the membranes were implanted under sterile conditions in a subcutaneous pocket made upon incision in the subscapular region. The wound was stitched with surgical threads. Each group was then divided into 3 sub-groups (n=3) according to different time point, the animals being euthanized after 10, 20 and 30 days by an overdose of a ketamine and xylazine mixture. The tissue containing the implanted membranes and the surroundings was explanted and fixed in 4% formalin for 24 h and subsequently dehydrated in a series of increasing alcohol concentrations and embedded in paraffin. Histological sections were prepared with a thickness of 3-5 μm and stained with hematoxylin and eosin (H&E) for histological evaluation. The histological images were captured by using Olympus CX40 microscope (Tokyo, Japan) equipped with photo camera Hitachi CCD and CellSens software. The histomorphometric measurements were performed in order to evaluate the extent and deep of the inflammation as a result of subcutaneous membrane implantation by comparison with the naturally healed tissue (control). The values were graphically represented as mean values ± standard deviation using the software GraphPad Prism 8.0 (statistical significance $p<0.001$ and $p<0.01$).

3. Results and discussions

3.1. Structural and morphological characterisation

In order to evaluate the incorporation of azelaic acid in the collagen matrix and subsequent possible modification of the structure, the FTIR spectrum of azelaic acid- collagenic membrane was recorded and compared with the pure collagenic membrane, along with the spectrum recorded for azelaic acid powder and presented in Fig. 1 (A, B, C).

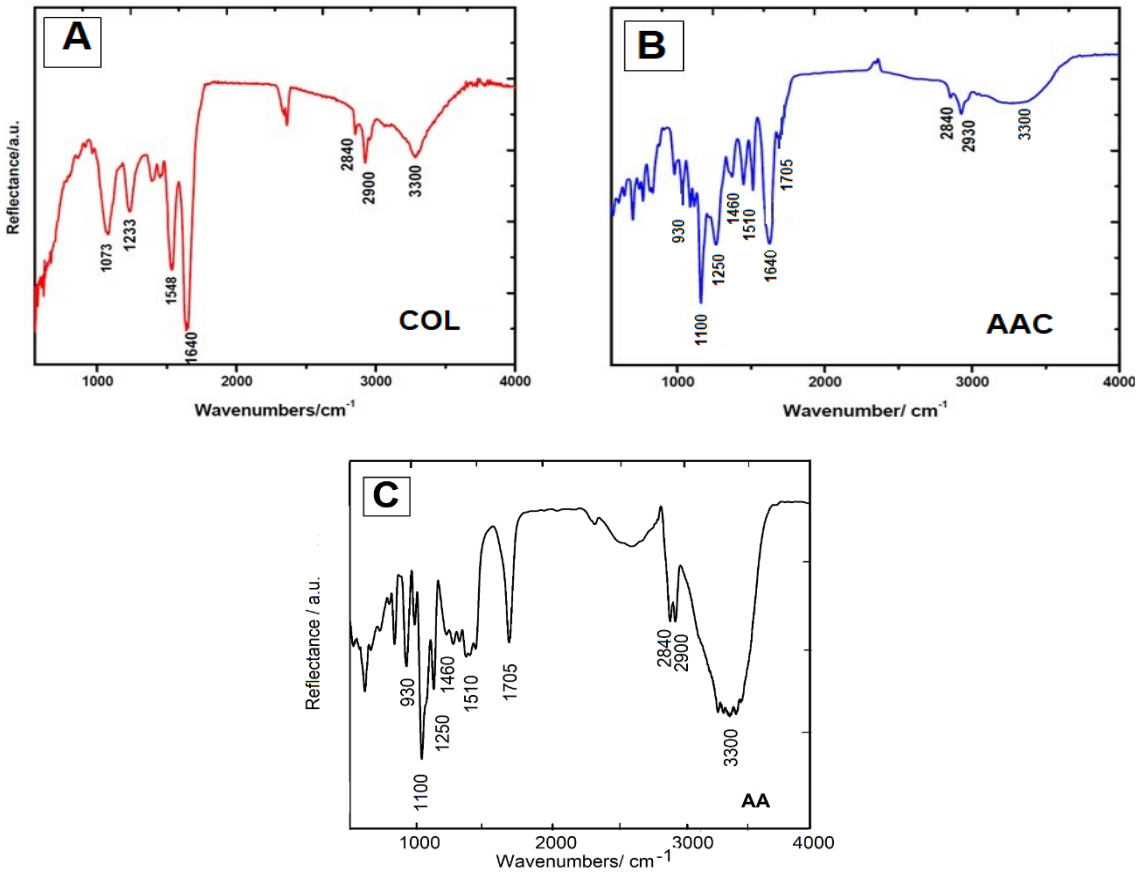

Fig. 1. (A) FTIR spectra of pure collagen membrane (COL); (B) azelaic acid-collagenic membrane (AAC); (C) pure azelaic acid in powder form, as provided by the supplier (AA).

The vibrational fingerprint of collagen (Fig. 1A) can be observed as characteristic bands at 1640, 1540 and 1233 cm^{-1}, attributed to amide I, II and III bands, respectively. According to literature, amide I bands originated from C=O stretching vibrations coupled to N-H bending, amide II bands results from the N-H bending coupled with C-N stretching vibrations, while amide III represent the combination between N-H deformation and C-N stretching vibrations [21]. The vibrational peak at 1073 cm^{-1} is due C-O-C vibrations. In the high wavenumber region, the characteristics of amide A and B, at 3300 cm^{-1} (-OH bending) and 2900 cm^{-1} (NH stretching) can be observed. The FTIR spectrum of the azelaic acid-collagenic membrane shows the same vibrational characteristic of the parent molecules, with only minor changes. It can be noticed that the marker bands of azelaic acid are well preserved in the collagenic matrix (Fig. 1B and Fig. 1C), indicating that this molecule remains stable upon the incorporation procedure. Azelaic acid possesses two carboxylate groups with the fingerprints at 724, 930, 1250, 1510 and 1705 cm^{-1}(very strong), and a pair at 2840/2900 cm^{-1}, these features being reported by other studies as well [22]. Minor modifications in the features of azelaic acid-collagenic membranes versus pure collagen were noted in terms of relative intensity of the marker bands and slightly shift of amide II and amide III peaks position.

All these spectral features demonstrates that azelaic acid was successfully incorporated and well preserved in the collagen matrix, while the matrix act as a reservoir for possible release of the bioactive molecules under physiological conditions.

In order to investigate the ultrastructural details of membranes, SEM images were recorded on the surface and cross-sectioned area, and presented in Fig. 2.

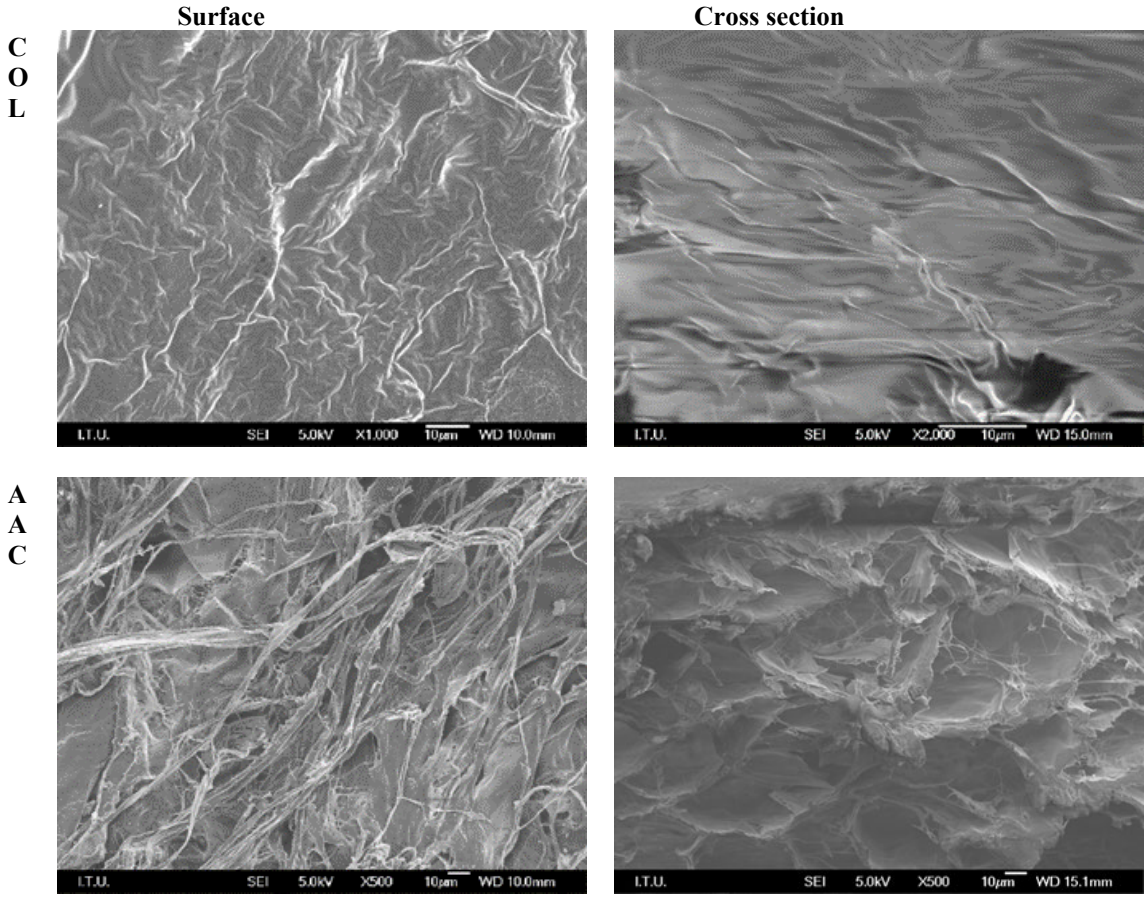

Fig. 2. SEM morphological details of the pure collagen membrane (COL) and azelaic acid collagenic membrane (AAC) recorded on the surface and cross section.

Comparing the morphological details, one can observe very different ultrastructural features between the two membranes. It can be noticed a dense and compact structure of pure collagen membrane (COL), both on the surface and cross section area. On contrary, the AAC membrane presents a fibrillar surface structure with distinct collagen fibers, randomly oriented, while on cross section view, a porous structure can be observed, with a tendency to adopt a honeycomb architecture. Similar network of collagen fibers bundles was evidenced in commercial Biocollagen® membranes designed for guided bone regeneration [2]. All these ultrastructural details might have a crucial importance, by influencing the resorption process and the immune response [23]. It is generally accepted that porous structure is favourable for soft tissue regeneration applications, promoting re-epithelialization and neovascularization [24, 25].

3.2. Nanoindentation measurements

Load-displacement curves and the Young modulus values corresponding to pure collagen membrane and azelaic acid collagenic membrane are presented in Fig. 3 (A ,B)

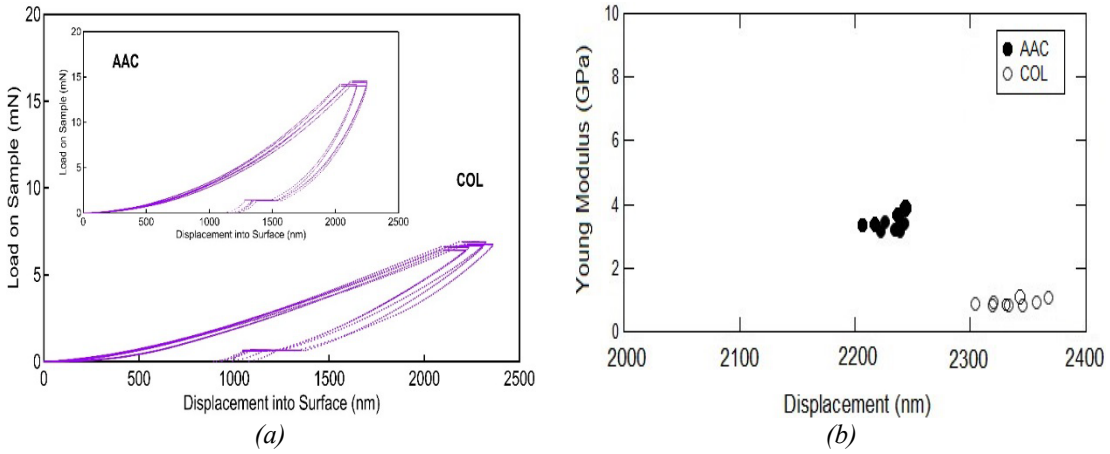

Fig. 3. (A) Nanoindentation curves recorded on the surface of collagenic membrane (COL) respectively azelaic acid collagenic membrane (AAC- inset) and (B) corresponding Young modulus values.

As observed in the load-displacement profile (Fig. 3A), a significant modification occurred when azelaic acid was incorporated in the collagenic matrix. In this case, the applied load was almost double in order to reach the same nano-indentation. In terms of Young modulus values, we noticed an increased from E=1.2 ± 0.1 GPa to E=3.9±0.2 GPa when azelaic acid was incorporated in collagenic matrix. Comparing with available data reported in literature [26,27], we found that the average value of Young modulus of a single collagen fiber is between 1.2–2 GPa. It is well known that the triple helix within the collagen molecules is stabilized by hydrogen bonds, which confer the mechanical strength of a single collagen fiber. When collagen fibers are assembled in a membrane, higher Young modulus values are expected. However, very different values were calculated for several collagenic membranes manufactured by Jason®, CovaTM Max, Biocollagen®, as reported by Ratiu et al [2]. In this case, the authors demonstrated a slightly increased stiffness of commercial membranes after PRGF (plasma rich in growth factors) treatment, designed for guided bone regeneration. However, it is generally accepted that any chemical, enzymatic or mechanical treatment applied to natural collagenic membranes may retain the mechanical properties of the native collagen and subsequent promote tissue remodelling by neovascularization and recellularization [28,29].

3.3. In vivo tests. Histopathological analysis and histomorphometrical results

The subcutaneous animal model was chosen in this study because of its ease in manipulation and the low cost of experimental procedures.

Overall, pre-and postoperative procedures were very well tolerated by the animals and no complications were noted.

The histopathological images of the explanted membranes and the surrounding tissue collected at each time point (10, 20 and 30 days) are presented in Fig. 4, in comparison with the tissue without implanted membrane, which was allowed to heal naturally.

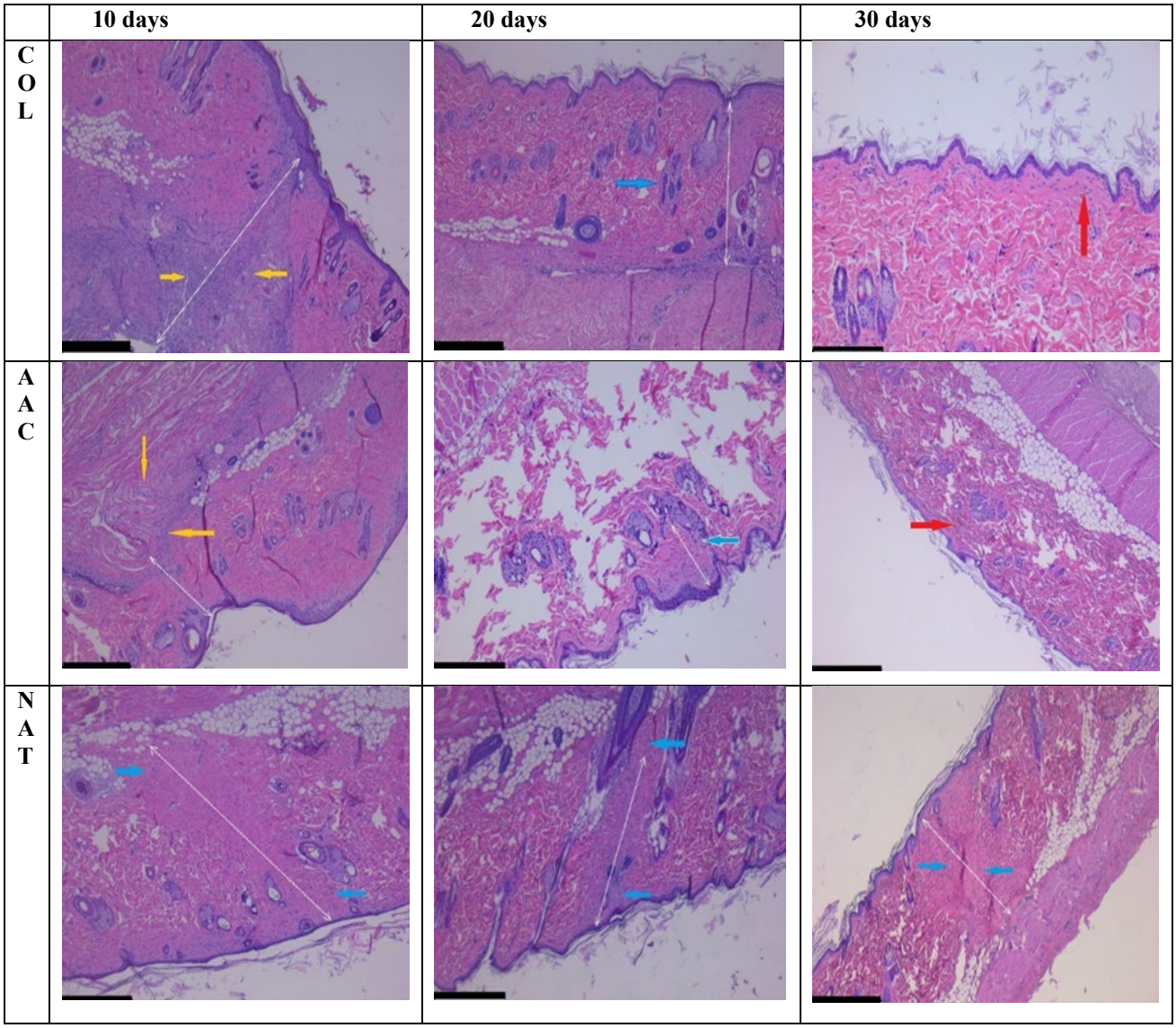

Fig. 4 Histological images of subcutaneous tissue at different time points (10, 20 and 30 days) after sample implantation: COL – pure collagenic membrane; AAC – azelaic acid collagenic membrane; NAT – naturally healed tissue, following the surgical incision. The distances highlight the size of the inflammation. H&E staining. Objective 20x; scale 450 µm.

After the first 10 days, as a result of subcutaneous implantation, an inflammatory reaction can be noticed in all cases, accompanied by proliferation of endogenous collagen fibers and fibroblasts, within the normal healing process (blue arrow). The reactive tissue does infiltrate the membranes in both cases (COL and AAC), but with different degrees. The reaction is minimal in the case of AAC sample, the regeneration process being visible even in this early stage. In the control sample (NAT) one can observe an intense inflammatory process at the level of profound cutaneous tissue, with obvious tendency to abscess, displaying extensive fibrous tissue with thick collagenic fibers oriented in perpendicular plane. The local inflammation and fibrous tissue extended from the epidermic level to hypoderm, near the limit to the muscle layer, as pointed out by the two blue arrows, while the fragments of implanted collagenic membranes are evidenced by yellow arrow.

After 20 days post-implantation, the membrane COL was still present in the subcutaneous tissue, but the inflammatory reaction was significantly reduced, and a minimum area of fibrous regeneration can be noticed, orientated perpendicular to the epidermal layer. On contrary, AAC membrane was completely resorbed within the tissue. Endogenous collagen fibers and fibroblasts

can be observed within the process of tissue remodelling. In the control sample (NAT) extensive fibrous tissue can be noticed, from the epidermic level to hypoderm, in contact with pilous follicles (blue arrow). Both membranes (COL and AAC) were completely resorbed after 30 days, while the inflammatory reaction gradually diminished until completely healing. No signs of necrosis or foreign object were observed [30].

By comparatively analysing the evolution of tissue regeneration and immune response, we appreciate the AAC membrane presented a favourable response and good bio-integration since the first 10 days, in contrast to COL and NAT sample, which generated relative intense inflammatory process. Moreover, the resorption of AAC membrane occurs in a shorter time compared to COL. The red arrows highlight the *ad integrum* regeneration area after 30 days.

The results of the histomorphometric measurements are presented in Fig. 5

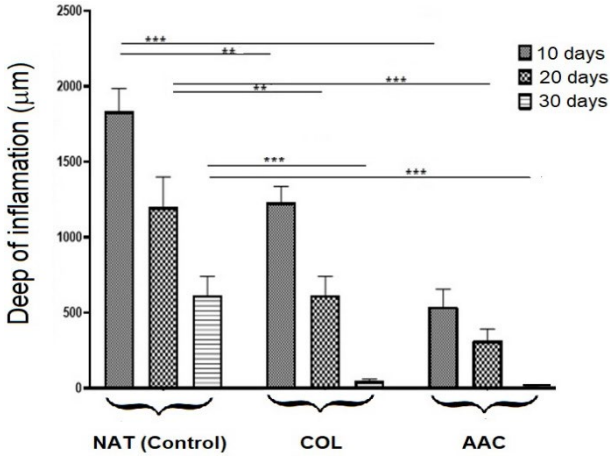

*Fig. 5. Histomorphometric measurements representing the deep of inflammation and evolution in time, as a result of subcutaneous implantation of COL and AAC membranes, compared to the natural healing of the tissue (NAT). Statistical significance ***p<0.001, **p<0.01.*

These findings can be compared with similar results reported in literature. For example, Radenkovic et al [31] elaborated an *in vivo* model in order to compare the bio-integration capacity of different commercial collagenic membranes designed for tissue regeneration. All the tested membranes were porcine-derived sugar crosslinked collagen membranes, prepared by different cross-linking procedure, and hence, with different structural characteristics, respectively compact or porous structure. Through histological analysis, the authors evidenced a lacked in cellular infiltration and trans-membranous vascularization of the compact structured-membrane, being almost intact after 60 days implantation, while the porous membrane was able to gradually integrate in the surrounding tissue, with complete resorption after 60 days. Similar results were reported by Neto et al [32], comparing the tissue reaction and the time of degradation of two commercial absorbable membranes: a bovine collagen membrane (Lyostypt®) and a porcine collagen membrane (Bio-Gide®) implanted in the subcutaneous tissue of mice. The authors demonstrated that the biodegradation of bovine-derived collagenic membrane occurred more rapidly compared to the porcine-derived one (after 60) days, but however they concluded that both membranes were considered biocompatible since their tissue reactions were compatible with the physiological inflammatory process.

Our results are in line with these previous findings, suggesting that the bio-integration process and immune response depends significantly on the ultra-structural details of the membrane and the provenance of the collagen. Moreover, the anti-inflammatory effect of azelaic acid was once again demonstrated. Recently, a novel liposomal formulation with azelaic acid was reported [18], evidencing the protective effect of azelaic acid against hydrogen-peroxide-induced DNA damage in fibroblasts. In this context, our results might have a significant importance, as the new developed

membrane can provide the desired standing time required for wound healing, being a promising option for larger tissue defects, protecting the wound from bacteria and facilitating the healing process.

4. Conclusions

In the present work, we have successfully prepared collagenic membranes with azelaic acid incorporated for possible wound healing applications. As demonstrated by FTIR spectra, azelaic acid was very well incorporated and preserved in the collagenic matrix. The ultrastructural details evidenced different features between pure collagenic membrane and azelaic acid-collagenic membrane: a porous structure with a honeycomb-style architecture was achieved as a result of azelaic acid incorporation in collagen membrane. Moreover, a slightly increased stiffness was noticed as a result of azelaic acid incorporation, as demonstrated by nanoindentation measurements. Based on *in vivo* tests, we suggest that the bio-integration process and immune response depends significantly on the ultra-structural details of the collagenic membrane. Upon azelaic acid incorporation, the resorption of collagenic membrane occurs in a shorter time compared to pure collagenic one, with only minor inflammatory events. Being well known the antibacterial and anti-inflammatory effect of azelaic acid, our results might have a significant importance, as a promising option for healing the deep and large cutaneous tissue defects.

Funding

This research was funded by University of Oradea, Romania.

References

[1] C. Lindner, S. Alkildani, S. Stojanovic, S. Najman, O. Jung, M. Barbeck, Membranes 12, 378 (2022); https://doi.org/10.3390/membranes12040378

[2] C. Ratiu, M. Brocks, T. Costea, L. Moldovan, S. Cavalu, Appl. Sci. 9, 1035 (2019); https://doi.org/10.3390/app9051035

[3] R. Sklenářová, N. Akla, M. J. Latorre, J. Ulrichová, J. Franková, J. Funct. Biomater. 13, 249 (2022); https://doi.org/10.3390/jfb13040249

[4] G. Tronci, Advanced Textiles for Wound Care, 2nd Edition, Woodhead Publishing (2019) Chapter 13, p. 363-389; https://doi.org/10.1016/B978-0-08-102192-7.00013-8

[5] F. Miere (Groza), A.C. Teușdea, V. Laslo, S. Cavalu, L. Fritea, L. Dobjanschi, M. Zdrinca, M. Zdrinca, M. Ganea, P. Pașc, A. R. Memete, A. Antonescu, A.M. Vlad, S.I. Vicas, Appl. Sci. 11, 11526 (2021); https://doi.org/10.3390/app112311526

[6] S. Cavalu, P. M. Pasca, M. Brocks, Materiale Plastice, 56, 3, 479-483 (2019); https://doi.org/10.37358/MP.19.3.5213

[7] Dau, M.; Volprich, L.; Grambow, E.; Vollmar, B.; Frerich, B.; Al-Nawas, B.; Kämmerer, P.W. J. Biomed. Mater. Res. Part A 108, 2368-2378 (2020); https://doi.org/10.1002/jbm.a.36989

[8] Y. Wu, S. Chen, P. Luo, S. Deng, Z. Shan, J.Fang, X. Liu, J. Xie , R. Liu , S.Wu , X. Wu, Z. Chen , K. W.K. Yeung , Q. Liu , Z. Chen. Acta Biomaterialia 143, 159-172 (2022); https://doi.org/10.1016/j.actbio.2022.02.004

[9] S. Cavalu, V. Simon, G. Goller, I. Akin. Digest Journal of Nanomaterials and Biostructures 6, 2, 779-790 (2011).

[10] D.M. Verissimo, R.F. Leitao , R.A. Ribeiro , S.D. Figueiro , A.S. Sombra , J.C. Goes , G.A. Brito, Acta Biomaterialia 6, 10, 4011-4018 (2010); https://doi.org/10.1016/j.actbio.2010.04.012

[11] R. Yu, H. Wang, R. Wang, P. Zhao, Y. Chen, G. Liu, X. Liao. Water Res. 30, 218, 118469 (2022); https://doi.org/10.1016/j.watres.2022.118469

[12] H.-I.M. Ibrahim, M. Thangavelu, A. Khalifa. Molecules 27, 7090 (2022); https://doi.org/10.3390/molecules27207090

[13] P. M. Pasca, S. Cavalu. Digest Journal of Nanomaterials and Biostructures 16, 3, 929 - 938 (2021); https://doi.org/10.15251/DJNB.2021.163.929

[14] H. Liu, M. Lin, X. Liu, Y. Zhang, Y. Luo, Y. Pang, H. Chen, D. Zhu, X. Zhong, S. Ma, Y. Zhao, Q. Yang, X. Zhang. Bioactive Materials 5, 4, 844-858 (2020); https://doi.org/10.1016/j.bioactmat.2020.06.005

[15] A. Ehterami, M. Salehi, S. Farzamfar, A. Vaez, H. Samadian, H. Sahrapeyma, M. Mirzaii, S. Ghorbani, A. Goodarzi. Int. J. Biol. Macromol. 117, 601-609 (2018); https://doi.org/10.1016/j.ijbiomac.2018.05.184

[16] J.G. Min, U.J. Sanchez Rangel, A. Franklin, H. Oda, Z. Wang, J. Chang, P.M. Fox. Antimicrob. Agents Chemother. 64(10):e00136-20 (2020); https://doi.org/10.1128/AAC.00136-20

[17] T. Ahmad, S. McGrath, C. Sirafim, R. do Amaral, S.L. Soong, R. Sitram, S. Turkistani, F. Santarella, C.J. Kearney. Biomater. Sci. 9, 4278-4288 (2021); https://doi.org/10.1039/D0BM01277G

[18] P.M. Pasca, F. Miere (Groza), A. Antonescu, L. Fritea, F. Banica, S.I. Vicas, V. Laslo, D. C. Zaha, S. Cavalu. Appl. Sci. 12, 13039 (2022); https://doi.org/10.3390/app122413039

[19] A. Kumar, R. Rao, P. Yadav. Current Drug Therapy 15(3) 181-193 (2020); https://doi.org/10.2174/1574885514666190904160228

[20] C. Charnock, B. Brudeli, J. Klaveness. European Journal of Pharmaceutical Sciences, 21(5) 589-596 (2004); https://doi.org/10.1016/j.ejps.2003.12.006

[21] B. de Campos Vidal, M. L. S. Mello. Micron, 42, 283-289 (2011); https://doi.org/10.1016/j.micron.2010.09.010

[22] P.-W. Hsieh, S.A. Al-Suwayeh, C.-L. Fang, C.F. Lin, C.C. Chen, J.-Y. Fang. Eur. J. Pharm. Biopharm. 81, 369-378 (2012); https://doi.org/10.1016/j.ejpb.2012.03.006

[23] M. Barbeck, J. Lorenz, M.G. Holthaus, N. Raetscho, A. Kubesch, P. Booms, R. Sader, C. J. Kirkpatrick, S. Ghanaati. J. Oral Implant. 41, e267-e281 (2015); https://doi.org/10.1563/aaid-joi-D-14-00155

[24] R. Sridharan, A. Cameron, D. Kelly, C. Kearney, F. J. O'Brien. Mater. Today 18, 313-325 (2015); https://doi.org/10.1016/j.mattod.2015.01.019

[25] S. Cinta-Pinzaru, S. Cavalu, N. Leopold, R. Petry, W. Kiefer. J. Mol. Struct. 565, 225-229 (2001). https://doi.org/10.1016/S0022-2860(00)00930-3

[26] S.V. Kontomaris, D. Yova, A. Stylianou, G. Balagiannis. Scanning 37, 101-111 (2015); https://doi.org/10.1002/sca.21185

[27] S.-H Lee, S.-J. An, Y.-M. Lim, J.-B. Huh. Materials 10, 1018 (2017); https://doi.org/10.3390/ma10091018

[28] A. Stylianou, D. Yova. Mater. Sci. Eng. C, 33, 2947-2957 (2013); https://doi.org/10.1016/j.msec.2013.03.029

[29] Y.-Z. An, Y.-K. Kim, S.-M. Lim, Y.-K. Heo, M.-K. Kwon, J.-K. Cha, J.-S. Lee, U.-W. Jung, S.-H. Choi. Dent. Mater. J. 37, 332-340 (2018); https://doi.org/10.4012/dmj.2017-065

[30] S. Cavalu, V. Simon. J. Optoelectron. Adv. Mater. 8, 4, 1520-1523 (2006).

[31] M. Radenković, S. Alkildani, I. Stoewe, J. Bielenstein, B. Sundag, O. Bellmann, O. Jung, S. Najman, S. Stojanović, M. Barbeck. Membranes 11, 712 (2021); https://doi.org/10.3390/membranes11090712

[32] A.M.D. Neto, S.C. Sartoretto, I.M. Duarte, R.F. d.B. Resende, A.T. Neves Novellino Alves, C.F.d.A.B. Mourão, J. Calasans-Maia, P. Montemezzi, G.C. Tristão, M.D. Calasans-Maia. Membranes 10, 423 (2020); https://doi.org/10.3390/membranes10120423