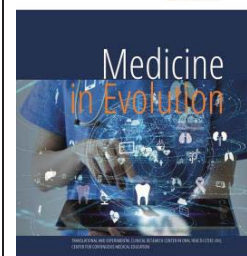


# Applications of Human Amniotic Membrane in Oral Medicine: Literature Review



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## Abstract

The Regenerative Medicine has become a contemporary reality, which is why the current research in this field is no longer and attractive idea, but a veritable demonstrated truth. It includes a number of technologies based on the biology and physiology of embryonic stem cells and human amniotic membrane (hAM) transplantation. Aim and objectives: The aim of the study is to summarize the way in which pathologies with oral manifestations were treated by applying different forms of hAM. The properties of the hAM described over time and subject to many contradictions of interpretation, thus acquire a practical foundation, being supported only by the results extracted from clinical studies carried out on human subjects. Material and Methods: Following the research, 20 scientific articles were included. After applying the exclusion criteria, 9 articles remained. The following parameters were monitored postoperatively: secondary inflammation, the return degree of the sensory response of the tissues to physiological parameters, secondary allergic reactions, the coverage degree of the root surfaces and the epithelization effect. Results: hAM was used in 82 patients with variable age ranges (20-85 years old). After the surgery, there were identified: the absence of secondary inflammation, a physiological sensory response of the tissues and the absence of allergic reactions in all cases. The degree of root coverage was 100%. The epithelization effect was good. The galenic form of hAM identified in all 9 studies was represented by the tissue film (patch). Conclusions: hAM has been successfully used in reconstructive surgery protocols. It's properties can be defined as theories with a proven medical foundation, even if the number of relevant studies in this study is not large and the number of patients is relatively small.

**Keywords:** human amniotic membrane, reconstructive surgery, regenerative medicine

## INTRODUCTION

Today, the regenerative medicine includes a number of technologies based on the biology and physiology of the human amniotic membrane. This offers hope for the preservation of the functions of the organ systems and mainly the functions of the stomatognathic system.

The human amniotic membrane is defined as the internal layer of the fetal membranes, making up, by its extension and distribution, the internal wall of the amniotic sac [1]. The human placenta can be easily collected from natural births or during caesarean surgery.

Over time, studies have demonstrated that the amniotic membrane possesses a series of characteristics of utmost importance, these being divided into 2 large categories:

biological properties and mechanical properties [2].

Table 1. Biological and mechanical properties of hAM

Biological properties	Mechanical properties
■ Immunogenic capacity	■ Elasticity
■ Antifibrotic effect	■ Rigidity
■ Anti-inflammatory effect	■ Dimensional stability
■ Epithelization effect	■ Permeability

The first attestation regarding the use of amniotic membrane in the medical field dates back to 1910 when Davis and his collaborators [3] used hAM as a graft to restore an area of skin. In the field of oral medicine, the application of the amniotic membrane was mentioned only in 1985 by Lawson et al. who described a specific protocol for tissue reconstruction of the oral mucosa by a histological combination of autogenous striated muscle tissue from the pectoralis major muscle and amniotic membrane graft [4]. Subsequently, hAM subjected to electron microscopy studies was quickly introduced on the list of oral mucosa substitutes, to which it resembles cellularly and molecularly [5].

A number of in vivo or in vitro studies on laboratory animals have been carried out over time, but the results obtained over time have been contradictory. Moreover, the data from the literature were supplemented with results obtained on human cells but through in vitro procedures. Thus a large number of contradictions considering the regenerative capacities of the tissues and the effectiveness of hAM have appeared.

### *Aim and objectives*

Starting from the discrepancies encountered in preclinical studies, with minor statistical relevance, this article brings to light the results obtained by using amniotic membrane in clinical studies on human subjects. Over time, hAM has been successfully used in the surgical treatment of numerous mucosal and muco-gingival conditions and deformities: precancerous lesions [6], benign tumors and malignant tumors. Moreover, the inclusion of hAM allograft in the plasty of defects resulting from tonsillectomy represented an important step in ENT (ear, nose and throat) surgery [7].

The purpose of our review consists in presenting some data from the literature, in which the properties of hAM are theoretically strengthened by its practical applicability on different lesions in oral cavity.

## MATERIAL AND METHODS

### *Data collection*

A number of articles were searched in the most widely used medical content databases worldwide. Among the databases used we list: PubMed, Wiley Online Library, Google Scholar and Scopus. In order to obtain an up-to-date documentation, articles and specialized magazines published between the years 2013-2024 were researched. Thus, notions such as: ("human amniotic membrane", "properties of amniotic membrane", „amniotic stem cells") AND ("amniotic membrane in dentistry", "amniotic membrane in oral pathology", "amniotic membrane in oral surgery") were introduced. Following the research, in the current study, 20 scientific articles were taken into account in the first stage.

### *Inclusion / exclusion criteria*

The inclusion criteria included three categories of clinical trials: prospective clinical trials, retrospective clinical trials and case-report studies. Regarding the diagnostic category, the inclusion criteria strictly included primary oral pathologies or with secondary manifestations in the human oral cavity. Exclusion criteria applied to the 20 articles were: research on laboratory animals, in vitro research and studies on the use of amnion allografts in other medical specialties. As such, from the total number of studies, 5 articles were excluded with reference to the study on laboratory animals, 4 studies with the applicability of amnion allografts in pathologies without manifestations in the oral cavity and 2 studies carried out in vitro.

The information collected from the 9 publications is summarized in: the number of human subjects involved in the study and/or their sex, the oral pathology of the patients and the evaluation criteria of the effectiveness of the allograft over a certain period of time.

## RESULTS

In order to achieve a logical approach to extracting the necessary information, the studies were divided into 5 different diagnostic areas belonging to specialties such as oral and maxillofacial surgery and periodontal surgery: benign (and/or cystic) oral lesions, malignant oral lesions, oro-sinusal pathological communication, jaw induced osteonecrosis and gingival recessions.

### ORAL AND MAXILLO-FACIAL SURGERY

#### *◇ Benign (and/or cystic) oral lesions*

In the study conducted on a number of 5 subjects with an average age between 36-75 years, Amemya et al. investigated for a postoperative period of up to 12 months the possibility of infection, secondary hemorrhages and the degree of immunological rejection following the application of hAM after the excision of 3 mucous cysts located at the labial level and a pleomorphic adenoma of the upper lip. The results obtained were impressive, with patients not complaining of symptoms characteristic of secondary inflammation or bleeding at the site of membrane application [7].

The clinical study of Srikant et al. was carried out on a group of 15 patients (12 men and 3 women) aged between 23-65 years. They followed the degree of epithelialization of the oral mucosa after the application of the amniotic membrane on the postexcisional defects of some lesions, one of the patients presenting a capillary hemangioma of the labial mucosa [9]. In addition to the degree of epithelialization identified as variable and in the study by Amemiya et al., Shrikant et al. follow in their research a number of other parameters such as: the pain, the sensory response of the affected area, the opening degree of the oral cavity and the cicatricial contraction phenomenon. The postoperative pain was imperceptible a few days after the surgical interventions and the sensory response of the tissues became physiological

again in 100% of the cases. Moreover, the degree of physiological opening of the oral cavity obtained a high percentage at 7 days (85% of cases) and a score of 100% at two weeks postoperatively. Scar contraction, from the mentioned data, was identified in only one case, which could be attributed to a larger lesion [9].

◊ *Malignant oral lesions*

In the prospective clinical study carried out by Bijan Khademi et al., amniotic membrane patches were sutured to tissues after the removal of primary malignant tumors located on the tongue, hard and/or soft palate, as well as in the mandibular retromolar space. The study included a number of 50 patients (40 men and 10 women), with an average age between 20 and 80 years, who underwent partial glossectomy interventions, partial oral floor resections or even partial mandibular bone resections [10]. Following the histo-pathological examination, the cancerous lesions proved to be squamous cell carcinomas.

No patient suffered allergic reactions, which demonstrates the immunomodulatory capacity of hAM [11,12,13].

Deep tumors of the oropharyngeal space, with invasion of the mandibular bone surfaces, were also identified at patients in the study published by Ann Kakabadze et al. in 2017. In their retrospective clinical study, 4 subjects diagnosed with malignant tumors of conjunctival origin (osteoblastoma and osteosarcoma with osteoblastic cells), underwent resection interventions between 2008 and 2015, reconstructive plasty being subsequently performed through different clinical protocols [14]. By comparison, the patients were treated as follows: 3 patients benefited from the application of a biomaterial composed of bone allograft (autogenous graft) from the rib with a mediator substrate of amniotic allograft and one patient benefited from the application of a biomaterial with the same type of mediator substrate but with a base consisting of biologically active bone graft taken from bovine femur [14]. It is noteworthy that the mediation between these addition biomaterials and the recipient tissues is nevertheless accomplished by hAM. This fact strengthens the immunomodulating property of the amnion upon contact with the host tissue, which was also demonstrated in Bijan Khademi et al. study [10].

In order to create a biologically active bone, which shows the ability of osteogenesis in the host area, the researchers (Ann Kakabadze et al.) composed a biomaterial from two components: a three-dimensional biological base of cancellous bone tissue taken from the bovine femur and a superficial component represented by stem cells of hematogenous red bone marrow harvested by aspiration from the patient's anterior superior iliac spine. The biomaterial was applied indirectly to the site of the injury, the interface between the acceptor area and the biomaterial being represented by hAM allograft. The idea of mediating interface through allograft also started from the researchers' precaution to cancel in any way the possible appearance of fibrous tissue at the transplant site. Thus, the antifibrotic capacity of the amniotic membrane was once again demonstrated [14].

Table 2. hAM applications in the surgery of benign and/or malignant tumors

The authors Year	Number of patients / Average age	Therapeutic indication (Pathology)	Parameters for evaluating the effectiveness of hAM	Results during the follow-up period
<i>Amemya et al. 2015</i>	5 patients /36-75 years old	Mucosal defects after excision of benign (and/or cystic) tumors	<ul style="list-style-type: none"> <li>▸ Degree of epithelialization</li> <li>▸ Hemostatic status</li> <li>▸ Degree of immunological rejection</li> </ul>	<ul style="list-style-type: none"> <li>▸ Complete epithelialization at 1 month in all cases</li> </ul>

<i>Kakabadze et al. 2017</i>	4 patients /38-55 years old	Muco-osseous defects after excision of malignant tumors	<ul style="list-style-type: none"> <li>▸ Radiologically detectable osteolysis</li> </ul>	<ul style="list-style-type: none"> <li>▸ Absence of complications in all cases</li> <li>▸ Radiologically detectable bone maintenance at 5 months</li> </ul>
<i>Shrikant et al. 2019</i>	15 patients /23-65 years old	Mucosal defects after extirpation of benign / premalignant tumors	<ul style="list-style-type: none"> <li>▸ Analgesia</li> <li>▸ Sensory response</li> <li>▸ Scar contraction</li> </ul>	<ul style="list-style-type: none"> <li>▸ Complete analgesia at 2 weeks</li> <li>▸ Normal sensory response in all cases at 6 months</li> </ul>
<i>Bijan et al. 2013</i>	50 patients /20-80 years old	Mucosal defects after extirpation of malignant tumors	<ul style="list-style-type: none"> <li>▸ Allergic reaction</li> </ul>	<ul style="list-style-type: none"> <li>▸ Lack of adverse effects in all cases</li> </ul>

◊ *Oro-sinus pathological communication*

Published in 2015, the study by Subha et al. describes an innovative method of plasty of an oro-sinus communication defect with dimensions of approximately 6 mm. Abandoning muco-periosteal flap therapy, the oro-antral communication that occurred after the extraction of a second upper molar in a 29-year-old female patient was resolved by suturing a patch of amniotic membrane stored at low temperatures of about 4 degrees Celsius [15]. The patient underwent a control period at intervals of 3, 5 and 7 days, tracking the amount of granulation tissue formed at the intervention site. After a period of two weeks, the epithelialization of the area was complete, the oro-sinus communication being closed.

◊ *Jaw induced osteonecrosis*

Bisphosphonates are widely used both in bone tissue pathology and as adjuvant medications in the chemotherapy of various malignant tumors [16]. The main disadvantage of using bisphosphonates is the occurrence of osteonecrosis of the jaw bones.

In the case-report study by Mirko Ragazzo et. al, a new therapeutic method is described, in which, the remaining defects after the debridement of areas with bone necrosis, were covered with hAM. The study included 2 patients of both sexes, aged 85 years, diagnosed with bisphosphonate-induced osteonecrosis in different regions of the facial massif and oral cavity. After surgery, patients were called for reevaluation at 180 days. Clinically asymptomatic, they did not show signs of local inflammation nor the appearance of secondary abscesses [16].

PERIODONTAL SURGERY

◊ *Gingival recessions*

Several authors wanted to demonstrate that hAM use in plastic techniques of denuded areas will have the effect of obtaining improved anatomical and structural configurations of the periodontal support apparatus. Among them, Mario Martelonni, Sonia Sheety and Anamika Sharma brought data to light.

In the case-report study published in 2015, Anamika Sharma and Komal Yadav look at the effectiveness and predictability of plasty of isolated areas of superficial-moderate root exposure using hAM. The study was carried out on a limited group of 3 patients, non-smokers, without associated pathologies, subjected to the preoperative scaling and root planing (SRP) procedure. They were classified according to Miller's classification in grade II canine gingival recession (at maxillary level). The denuded areas were treated by creating mucosal flaps (not including the periosteum), which covered amniotic membrane patches

placed in contact with the exposed areas. The values of parameters such as CAL (clinical attachment level) or RD (recession depth) decreased considerably for all 3 subjects participating in the study [17].

In the case report study published by Sonia Sheety et al. in 2014, gingival recessions due to root exposure were surgically treated by covering with hAM allograft and adjacent mucosal flap. The gingival recessions of the patient participating in the study were classified as Miller class I and were located at the level of several dental units (1.5 and 1.6, respectively 2.5 and 2.6). The study was carried out by comparison, the right side gingival recessions being surgically treated by plasty with Platelet-Rich Fibrin membrane (PRF) and covering mucosal flap and the left side recessions by plasty with hAM allograft and neighboring mucosal flap [18]. The comparative results between the methods indicated a maintenance of the degree of root coverage of 100% in both cases, highlighting a better dimensional stability of the postoperative areas for hAM allograft technique.

In the study published by Martelloni et.al, the case of a 40-year-old patient, classified in the same Miller class (class I), is presented. For 1.3 and 1.4 gingival recessions, the same bilaminar plasty technique was proposed (by covering with hAM allograft and relaxed and coronally translated vestibular flap). The results were identical to those presented by Sharma and Yadav [19].

The results obtained with using hAM as a tissue graft were satisfactory. The patients were not complaining of symptoms characteristic of secondary inflammation or infection at the site of hAM application (in all cases). The sensory response of the tissues became physiological again after the surgery (100%). Moreover, the degree of physiological opening of the oral cavity obtained a score of 100% at two weeks postoperatively. The values of parameters such as CAL or RD decreased considerably. A better dimensional stability of the postoperative areas was obtained when hAM allograft was used. No patient suffered allergic reactions.

## DISCUSSIONS

The purpose of this systematic review was to present how hAM allograft has been successfully used in a number of areas belonging to oral medicine.

Amniotic membranes were compared (in terms of effectiveness) with different biomaterials, existing in the pharmaceutical field at the time. Parallels were also made between the application of hAM allografts and the application of autogenous grafts from neighboring tissues.

hAM allograft, through its various uses, has virtually eliminated any doubt or uncertainty about its efficacy. This fact reinforces the idea that the biological and mechanical properties of hAM are not just formulated on paper, but are supported by a strong practical foundation. The anti-inflammatory effect was also promoted by Ragazzo et al. [16]. Moreover, this study also supports the theory of the analgesic and antimicrobial effect of hAM. The antifibrotic effect of hAM was found in the study by Shrikant et al., when hAM allograft opposed secondary fibrosis of the oral epithelium in patients suffering from submucosal fibrosis [9,20,21]. Bijan Khademi et al. complement the theories supporting the immunomodulatory property of hAM [10]. In their research, no subjects undergoing hAM reconstructive plastic surgery suffered allergic reactions [10,11,12,13]. In addition to the antifibrotic capacity, the study by Shrikant et.al, also highlights the analgesic effect of hAM [6].

The mechanical properties of the amniotic membrane played an equally important role, elasticity, tensile strength and dimensional stability being decisive in regaining the initial anatomical configuration of all tissues.

With all the progress made to date, the tissue engineering of amniotic membrane allografts deserves and needs to be further deciphered. We know that there is always hope for an even more resounding evolution in this field, considering the fact that, new applications of hAM in temporomandibular joint surgery and post-traumatic head surgery (orbital zone) have been described.

## CONCLUSIONS

Human Amniotic Membrane has been successfully used in reconstructive surgery protocols, being an easily accessible, biocompatible and qualitative graft from all points of view. This can function as a biological barrier against the aggression of antigens, thus increasing the chances of success of the restitutio ad integrum process. hAM stimulates postoperative wound healing by functioning as a protective, young and biologically integrated covering epithelium. The costs of collecting, processing and storing this allograft are minimal.

Although few in number, the disadvantages of using amniotic membrane should be remembered. The inconveniences of the amniotic membrane allograft collection process relate to the possibility of cross-infections in the presence of undiagnosed or unscreened donors for infectious pathologies, or to treatment and preservation procedures that do not respect the principles of asepsis and antisepsis.

In conclusion, the properties of the amniotic membrane can be defined as theories with a proven medical foundation, even if the number of relevant studies in our study is not large and the number of patients is relatively small.

### *Author contributions*

Conceptualization, A.U. and M.R.; Methodology, R.C.; Software, R.A.; Validation, B.I., D.C. and M.R.; Formal Analysis, A.U. and M.R.; Investigation, R.A.; Resources, R.C.; Writing – Original Draft Preparation, A.U.; Writing – Review & Editing, M.R. and R.A; Visualization, D.C.; Supervision, M.R.; Project Administration, B.I.

### *Conflicts of Interest*

The authors declare no conflict of interest.

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