



EVALUATION OF THE USING SOME BIOMATERIALS AS TISSUE CLOSING ADHESIVES AGENT: FIRST RESULTS
BAZI BİYOMALZEMELERİN DOKU KAPATMA YAPIŞTIRICISI OLARAK KULLANIMININ
DEĞERLENDİRİLMESİ: İLK SONUÇLAR

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ABSTRACT

Purpose: Synthetic glues like entellan are used for long-term preservation of tissues and procurement of brighter and clearer appearance under microscope. Generally, synthetic glues contain harmful chemicals such as methacrylate and derivatives. The toxic effects of synthetic polymeric adhesives on the environment and the wearer are known.

Material and methods: In this study, biomaterials of animal and plant origin were used to obtain glue mixtures. Polar solvent, citric acid, ammonia, hydrochloric acid, nano silica and clove oil were used in order to produce a more natural and healthy glue. Microscope images were obtained by closing the same tissues with mixtures containing biomaterials. Entellan was used as reference glue substance.

Results: The same image quality and closure strength were obtained with some mixtures according to tissues closed using entellan.

Conclusion: This first study will constitute a step to increase the use of natural glues containing biomaterials in dental and medicinal laboratories and other purposes since they have no pungent smell or toxic properties as well as being environmentally friendly.

Keywords: Entellan, natural biomaterials, natural glue, synthetic polymer, tissue mounting.

ÖZ

Amaç: Entellan gibi sentetik tutkallar, dokuları uzun süre korumak ve mikroskop altında daha parlak ve daha net görünmelerini sağlamak için kullanılır. Bu madde dokuya damlatıldıktan sonra üzerine bir lamel yerleştirilerek kapatılır. Sentetik tutkallar, metakrilat ve türevleri gibi zararlı kimyasallar içerir. Sentetik polimerik yapıştırıcıların çevre ve kullanıcı üzerindeki toksik etkileri bilinmektedir.

Materyal ve metod: Bu çalışmada tutkal karışımları elde etmek için hayvan ve bitki kaynaklı biyomalzemeler kullanıldı. Doğal ve sağlıklı bir tutkal üretmek için polar çözücü, sitrik asit, amonyak, hidroklorik asit, nano silika ve karanfil yağı gibi biyomalzemeler kullanıldı. Mikroskop görüntüleri, aynı dokuların biyomalzeme içeren karışımlarla kapatılmasıyla elde edildi. Referans tutkal maddesi olarak entellan kullanıldı.

Bulgular: Entellan kullanılarak kapatılan dokulara göre bazı karışımlarda aynı görüntü kalitesi ve kapanma mukavemeti elde edildi.

Sonuç: Bu ilk çalışma, biyomateryal içeren doğal tutkalların diş ve tıbbi laboratuvarlarda ve diğer amaçlarla kullanımını arttırmak için bir adım olacaktır. Çünkü keskin kokuları veya toksik özellikleri yoktur ve çevre dostudurlar.

Anahtar kelimeler: Doğal biyomalzemeler, doğal yapıştırıcı, doku kapatıcı, entellan, sentetik polimer.

INTRODUCTION

Polymers in organic structure are the raw materials of many industries. Adhesives have polymeric structure and are used in doors, windows, furniture, plastic containers, rubber, and drugs. One of the basic principles of organic chemistry, "like dissolves like" allows us to have an idea about the choice of solvent in advance (1,2).

Since polymers are apolar, they are diluted and dissolved using apolar solvents according to the chemical principle. However, the solubility of polyamide (PAM) polymers in different polar solvents was investigated and it was found that more PAM was solved in water than ethylene glycol (3). Thus, the solvent is used to hold the adhesives on the surface to which they are applied, not the bottle. When the adhesive is applied to the surface, the solvent in it evaporates and becomes thicker. Thus, the adhesive with increased viscosity hardens and adheres to the surface (4) if the solvent selected is a volatile substances such as alcohol, xylene, or chloroform instead of water, adhesion occurs in a shorter time and in the form of a tight connection (5). For this purpose, they are furniture, wood, felt, fabric, sponge surfaces, paper, and nylon materials. It is widely known that acrylic resin, methacrylate, and polyurethane based chemicals are used in adhesive substances (6,7). However, the use of adhesives to close tissues in medical laboratories and research centers such as pathology and histology departments in hospitals is not widely known. Here, first the biopsy samples are taken from the patients, tissue follow-up procedures are practiced and approximately 0.5 micron sections are cut with a microtome and transferred to slides, the sections are stained for microscopic observation, one drop is placed onto the entellan tissue, and covered with a coverslip (8-10). It is a product of Entellan® from Merck which is widely used in health and textile fields (11-14). Entellan methacrylate is a glue containing a toxic, malodorous solvent containing synthetic material. In the industry, as a natural sourced bioadhesive, plant products are resin, mucilage, gel etc. Parts are used mostly as paper and wood adhesive (15,16). Adhesives containing natural substances, which are healthier, non-toxic substances, and are solvent-free. Gelatine or dextran are produced for the school-age children to use (17,18). Gelatine, a formal-containing mixture is used in surgical medicine as a biological adhesive (19-21).

The aim of this study is to create non-toxic, environmentally friendly, easily supple and manufacturable adhesives containing not synthetic but natural materials.

MATERIALS AND METHOD

Biomaterials obtained from markets and florists of Talas region of Kayseri Province were quince, okra, aloe-vera, clove oil, and eggs. Gelatine, ammonia (NH₃), xylene, 70 % ethyl alcohol were used in analytical pure. The stained tissue preparates were obtained from idle rat tissues taken according to ethical standards

Tissue Supply

In this study, 1 Wistar albino female rat, weighing 150-250g and raised at the Erciyes University Experimental and Clinical Research Center (DEKAM) was used. This study was conducted with the ethics committee number

16/144 and dated 16 November 2016. The rat was kept in cages, at 21 C with normal daily organization, 12 h of light/dark, and was fed and watered appropriately. The rat was euthanized with ketamine (75mg/kg) and xylazine (10mg/kg) and its tissues taken. All of the procedures were performed according to ethical guidelines (22,23).

Preparation of Plant Extract Adhesive Compositions That Mounting Tissue

Seeds extracted from the quince fruit and okra were washed, shredded and placed separately in to the beaker in a ratio of 1: 2 (biomaterial gram: water ml) solution and kept at room temperature for three days. After filtering the solution with Whatman filter paper, 1 gram of citric acid was added to the resulting gel filtrates. After boiling for 5 minutes another extract, gels taken from the leaves of the aloe-vera plant and egg white were mixed by separately adding citric acid. The adhesives were boiled separately in two beakers for 10 minutes. The resulting mixtures were cooled and used as adhesive for mounting.

A gelatine solution was prepared by adding didistilled water to its yellow powder and boiled for 30 minutes. To the cooled 100ml filtrate 3 drops of clove oil was added. Four different mixtures (Gelatine¹, Gelatine², Gelatine³, Gelatine⁴) were prepared by adding 2 drops of NH₃ and 1 drop of HCl. The resulting mixtures were used separately to close the slides of the same tissue (Table I). Seven stained preparates from same tissue were used for dropping mixtures containing biomaterials. Photographs of the tissues on the slides are shown in 100x, magnification by light microscopy (Olympus BX -51, Japan).

RESULTS

Adhesives made with quince seeds, aloe-vera gel, egg white and okra biomaterials adhered to tissues, but a color appearance of the tissue was not achieved. The adhesive faded the color of the dye on the tissue. Purple staining of hematoxylin in the nucleus was not observed and the pink color in the cytoplasm was very pale. Therefore, microscopic observation results were not included here. However, the results of imaging of some tissues with Gelatine-contained mixtures yielded mounting positive and effective results with the preparations that were covered with entellan. The intensity of adhesion or mounting and microscopic exam were evaluated as negative (-) and positive (+) (in Table I).

Gelatine solution in water was used in the Gelatine 1, 2, 3 and 4 studies. However, the solution content was different in terms of added chemicals. Accordingly, the degree of adhesion between the lamel and lam observed by microscopic observation resulted in observed differences. Negative (-) images in figures IB and ID, which were not good in terms of microscopic observation, show changes in dye color and distortions in the tissue. The best adhesion degree and microscopic observations are shown in figures IA and IC. The results were evaluated based on the fact that the mixtures did not interfere with the tissue dye, did not disturb the tissue structure and thus a had good microscope appearance.

DISCUSSION

Since the synthetic materials used in the production of

Table I: Adhesive content and application results

| Biomaterials | Supplement Chemicals | Surface Adhesion | Microscopic Exam |
|-----------------------|--|------------------|------------------|
| Gelatine ¹ | SiO ₂ + NH ₃ + HCl+C.oil | + | + |
| Gelatine ² | SiO ₂ + HCl+ C.oil | + | - |
| Gelatine ³ | DDW+NH ₃ +HCl+C.oil | + | + |
| Gelatine ⁴ | DDW+ HCl+ C.oil | + | - |
| Quince | DDW +citric acid | + | - |
| Egg | DDW +citric acid | + | - |
| Okra | DDW +citric acid | + | - |

*DDW: Didistilled water, C.oil: Clove oil, HCl: Hydrochloric acid, NH₃: Ammonia, SiO₂: Silisium oxide (nano)

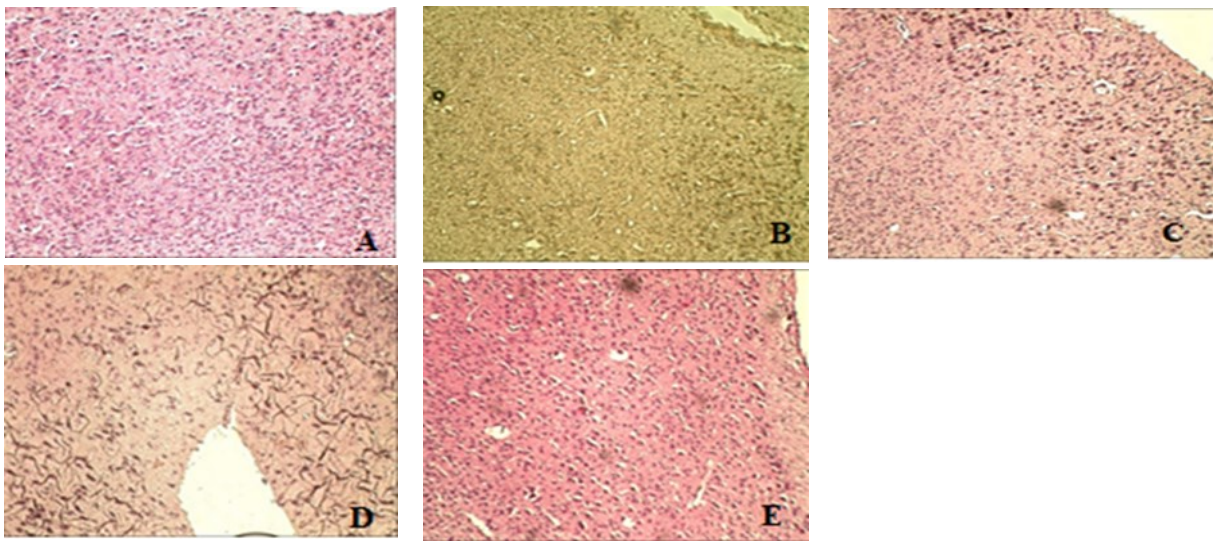


Figure I: Histological examination of different glue composition on the same tissue A) Gelatine¹ B) Gelatine² C) Gelatine³ D) Gelatine⁴ E) Entellan.

adhesives are generally polymeric, polymer structures found in the structure of plants and animals were used in this study (24). Gelatine is a protein powder obtained from animal bone. It is known to have cross-linked binding with tissue (25,26). Since it is an easily soluble substance in water, no distortion was observed for at least 6 months when 3 drops of clove oil were added to the solution prepared in a 1: 2 ratio. To this mixture 1 drop of NH₃ and 2 drops of HCl were added in order to get good examination. So that A good (+) color image of the tissue is shown in figure IC. When 0.01g of SiO₂ was added to mixture 3, good adhesion and good color image were obtained as shown in figure IA. Microscopic images and colors of non-NH₃ mixtures were observed to be distorted as in figures IB and D. Figure (E) provides a microscopic examination of the entellan covered tissue image commonly used in medical laboratories for comparison.

In this study, quince seed gel, okra juice gel, egg whites and aloe vera plant gel were used for their polysaccharide structures, i.e. polymeric structures. There are many plant, animal, and mineral origin substances but, the

colorful contents of herbal ones are used as a source of dye in the fields such as textile, food and medicine. There are many studies on the biological activities of the substances contained in plants (27-29). However, there are no studies in literature about natural glue for mounting tissue. This indicates the original value of the study. Entellan, is completely synthetic, has a pungent odor, contains harmful solvents, and is used in medical laboratories. However, the Gelatine 3,4 mixtures have different content biomaterials and work as environmentally friendly, non-toxic and completely healthy products. If some chemical and physical tests are done to study these mixtures, we can suggest the use of these mixtures in medical laboratories instead of entellan.. In addition, since the ingredients of these mixtures are very natural and harmless can be suggest that use for pharmaceutical purposes.

Disclosure statement

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Statement of Ethics

The author has no ethical conflicts to disclose.

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