ORIGINAL ARTICLES

STUDY OF PENETRATION ABILITY OF ADHESIVE SYSTEMS ON TEMPORARY TEETH BY CONFOCAL MICROSCOPY

Mamedov A.A.[1], Loschenov V.B.[2], Morozova N.S.[1], Ryabova A.V.[2], Ivannikova K.O.[1], Orlova N.A.[1]
[1]Sechenov First Moscow State Medical Univesity, Moscow, Russia
[2]Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow, Russia

Abstract

In this work, the authors evaluate the effectiveness of various adhesive systems (AS) in the temporary teeth restoration with composite materials. For this study, the authors used temporary teeth extracted for orthodontic reasons or lost due to a physiological change. Enamel samples with dentin were made from these teeth. Rhodamine B fluorochrome was added to each of the adhesive systems used. For the greater practical significance of research results, adhesive systems of several generations (IV, V, and VII) were chosen. In each sample, a cavity was prepared, and adhesive preparation was carried out according to the AS manufacturer instructions, followed by restoration with a composite material. Then, longitudinal slits were made so that the areas of enamel and dentin for which an adhesive protocol and restoration were performed could be observed in their entirety. The effectiveness of various generations was determined by the depth of penetration of AS components into the dentinal tubules. Fluorescent confocal microscopy was used to visualize the penetration ability of AS. Based on the results of the study, the authors concluded that IV and V generations of AS are the most effective.

Keywords: adhesive systems, dentin, dentistry, pediatric dentistry, confocal microscopy.

For citations: Mamedov A.A., Loschenov V.B., Morozova N.S., Ryabova A.V., Ivannikova K.O., Orlova N.A. Study of penetration ability of adhesive systems on temporary teeth by confocal microscopy, *Biomedical Photonics*, 2020, vol. 9, no. 2 , pp. 4–9. (in Russian) doi: 10.24931/2413–9432–2020–9–2–4–9.

Contacts: Morozova N.S., e-mail: kns74@bk.ru

ИССЛЕДОВАНИЕ ПЕНЕТРАЦИОННОЙ СПОСОБНОСТИ АДГЕЗИВНЫХ СИСТЕМ НА ВРЕМЕННЫХ ЗУБАХ МЕТОДОМ КОНФОКАЛЬНОЙ МИКРОСКОПИИ

А.А. Мамедов[1], В.Б. Лощенов[2], Н.С. Морозова[1], А.В. Рябова[2], К.О. Иванникова[1], Н.А. Орлова[1]
[1]Первый Московский государственный медицинский университет им. И.М. Сеченова, Москва, Россия
[2]Институт общей физики им. А.М. Прохорова РАН, Москва, Россия

Резюме

В работе представлены результаты исследования эффективности применения различных адгезивных систем (АС) при реставрации временных зубов композитными материалами. Для проведения исследования авторы использовали временные зубы, удаленные по ортодонтическим показаниям или утраченные вследствие физиологической смены. Из таких зубов были изготовлены образцы эмали с подлежащим дентином. В каждую из использованных АС был добавлен флуорохром (родамин В). Для большей практической значимости результатов были выбраны АС нескольких поколений: IV, V и VII. Далее в каждом образце отпрепарировали полость и провели адгезивную подготовку согласно инструкциям производителей соответствующих систем с последующей реставрацией композитным материалом. Затем для каждого образца изготавливали продольные шлифы с учетом полного попадания в область шлифа участков эмали и дентина, для которых проводили адгезивный протокол и реставрацию. Эффективность различных поколений определялась глубиной проникновения компонентов АС в дентинные трубочки. Для визуализации пенетрационной способности АС был использован метод флуоресцентной конфокальной микроскопии. По результатам исследования авторы делают вывод, что наибольшей эффективностью обладают АС IV и V поколения.

Ключевые слова: адгезивные системы, дентин, стоматология, детская стоматология, конфокальная микроскопия.

Для цитирования: Мамедов А.А., Лощенов В.Б., Морозова Н.С., Рябова А.В., Иванникова К.О., Орлова Н.А. Исследование пенетрационной способности адгезивных систем на временных зубах методом конфокальной микроскопии // Biomedical Photonics. – 2020. – Т. 9, № 2. – С. 4–9. doi: 10.24931/2413–9432–2020–9–2–4–9.

Контакты: Морозова Н.С., e-mail: kns74@bk.ru

Introduction

In modern restorative dentistry, composite materials are the materials of choice [1]. Their use has become widespread due to their strength and durability, as well as their user-friendly characteristics.

The success of the use of a specific composite material brand depends directly on the strength of its adhesion to hard dental tissues, enamel and dentin, and, therefore, only a well-chosen adhesive system (AS) can guarantee the stability of the restoration in the long term [2, 3].

Enamel and dentin processing performed before restoration can be divided into three stages: conditioning, priming, and bonding. At the first stage, orthophosphoric or organic acid is applied to the hard dental tissues, which decalcifies the intertubular and peritubular dentin, dissolves the smear layer formed during preparation, and opens the dentine tubules, which ensures the penetration of the components of the adhesive system into the thickness of the dentin. Then a primer is applied, which has hydrophilic groups that provide adhesion to dentine and hydrophobic groups that bind to the bond. The bond, also a component of the adhesive system, is applied after the primer, which provides adhesion to the composite material used for restoration [4].

Adhesive systems are classified into generations. 1st to 3rd generation systems are currently not in use. 4th generation AS are the most difficult to use: all three stages are separated, i. e., the conditioner, primer and bond are in separate vials. Further evolution of AS led to the integration of several processing stages into one. 5th generation AS have a separate enamel etching stage, but priming and bonding are combined. In the 6th generation systems, the conditioning stage is not separate, they are self-etching, and are supplied in two separate containers, requiring mixing before use. 7th generation is also self-etching, but with all stages combined into one [3]. Such adhesives are the easiest to use [5].

Full implementation of all the three stages of the adhesive protocol is difficult in the routine practice of a pediatric dentist [6]. Therefore, the most popular AS are self-etching ones and the systems where primer and bond are combined [7].

Over the past year, universal bonding systems have been gaining popularity. With them, the adhesive protocol is variable: depending on the clinical situation, the doctor decides whether to conduct the conditioning stage or not.

In this study, the authors compare the penetration ability of several kinds of the most popular AS using the method of fluorescent confocal microscopy.

Materials and methods

The study used intact primary masticatory teeth removed for orthodontic reasons or lost due to normal exfoliation. All parents of the patients signed informed voluntary consent to the use of primary teeth for the experimental study.

The primary teeth were used to make 63 samples of enamel with the subjacent dentin. Six AS most often used by pediatric dentists were selected for the study, two of which are universal. The adhesive protocol for each selected system was performed in accordance with the manufacturer's instructions.

The study used 4th generation AS (Bond A, 12 samples), 5th generation AS (Bond B, 10 samples), 5th generation AS (Bond C, 10 samples), 7th generation AS (Bond D, 11 samples), universal AS (Bond E, 10 samples) and universal AS (Bond F, 10 samples).

Additional characteristics of the studied AS are shown in Table 1.

Rhodamine B was selected as a fluorochrome, and its 0.01 wt-% solution was added to the bonding agents before their application [8].

The distribution of Rhodamine B fluorescence in tooth tissues was studied by laser scanning confocal microscopy with LSM-710 microscope (Carl Zeiss, Germany). To obtain images, a Plan-Apochromat lens with an X20 magnification (0.8 aperture) was used. For the study, tooth slices were placed on cover glasses with a thickness of 0.17 mm and observed in the plane of the cut. An argon laser with a wavelength of 458 nm (LASOS, Germany) was used to excite autofluorescence of dental tissues, and a DPSS laser with a wavelength of 561 nm (LASOS, Germany) was used to excite the fluorescence of Rhodamine B, in the ranges of 465 – 555 nm and 570 - 650 nm, respectively. Three-dimensional fluorescent images were obtained by registering a series of images with a step of 10 microns along the Z axis, followed by reconstruction of three-dimensional images with the use of ZEN software (Carl Zeiss, Germany).

The penetration capacity was determined by calculation of the average depth of penetration (l) and the number of filled dentine tubules in relation to all tubules in the field of vision (n) as a percentage in images obtained by fluorescent confocal microscopy:

$$n = (n_{fill}/n_{total}) \times 100\%$$
$$l = l_{ave}/n_{fill},$$

where n is the percentage of filled dentinal tubules among the total number of dentinal tubules in the field of vision;

n_{total} is the total number of dentinal tubules in the field of vision;

n_{filled} is the total number of filled dentinal tubules in the field of vision;

l is the penetration capacity;

l_{ave} is the average depth of penetration of the adhesive system into the depth of dentin.

Statistical processing of the data.

The exact confidence bounds to the frequency were calculated based on the binomial distribution. Student's t-test was used to determine the significance of the differences.

The protocol of sample preparation for the study

1. Since dentine tubes are directed centrically to the pulp chamber, the most suitable area for examination is the gingival margin of primary teeth. Cylinders containing enamel with the subjacent dentin are cut out from the gingival margin of the crown of a primary tooth, and then divided into 4 parts.
2. A cylindrical drill is used to produce a cavity in the center of the block, pertaining to class V in Black's classification of cavities.
3. Adhesive preparation of the sample is performed according to the manufacturer's instructions.
4. The cavity is filled with a flowable compomer.
5. The sample is cut in half.
6. Confocal microscopy of the sample is performed.

Results and discussion

Results are shown in Fig. 1 and 2.

A comparison of the effectiveness of individual systems representing various AS types, performed with the use of confocal fluorescence microscopy, brought the authors to the conclusion that the best penetration ability is observed in 4th and 5th generation AS. There was no statistically significant difference between them in the number of filled tubules in the field of vision of the obtained image, or in the depth of penetration. However,

Таблица 1
Сравнительная характеристика адгезивных систем
Table 1
Comparison of the adhesive systems

Наименование Name	Поколение Generation	Схема использования Scheme of using the adhesive system
Bond A	IV	3 этапа: кондиционирование; прайминг; бондинг 3 steps: conditioning; priming; bonding
Bond B	V	2 этапа: кондиционирование; прайминг+бондинг 2 steps: conditioning; priming+bonding
Bond C	V	2 этапа: кондиционирование; прайминг+бондинг 2 steps: conditioning; priming+bonding
Bond D	VII	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding
Bond E	универсальная система universal system	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding
Bond F	универсальная система universal system	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding

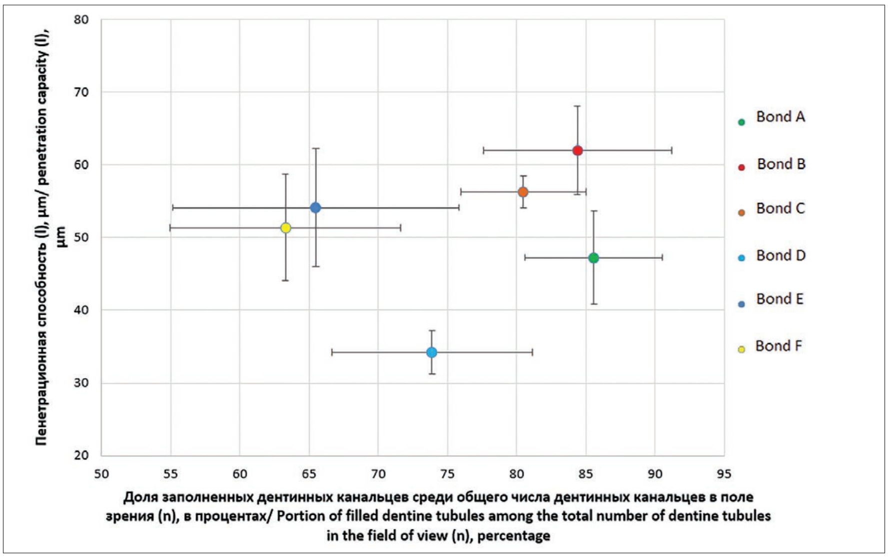

Рис. 1. Зависимость пенетрационной способности системы от доли заполненных дентинных канальцев
Fig. 1. Dependence of the penetration ability of the system on the fraction of filled dentinal tubules

significantly lower results were obtained for similar indicators in universal AS and 6th generation AS and universal bonding systems ($p<0.05$).

The results of this study can be explained as follows: during enamel and dentin preparation, a smear layer is inevitably formed on their surface, which acts as a barrier preventing AS components penetration into the thickness of the hard dental tissues [9]. However, etching with orthophosphoric acid at a concentration of 30-40% helps to remove the smear layer, providing microporosity of hard tissues and exposure of collagen fibrils [10]. Thus, the selected stage of conditioning contributes to the formation of a hybrid layer that is strongly integrated with dentin [11].

The results obtained by the authors are consistent with the data presented in the modern literature. T. Lemzi et al. [12] report in their literature review that AS with a separate conditioning stage show better results in comparison with self-etching AS. The authors made this conclusion based on an analysis of the results of *in vitro* studies, emphasizing that this fact could be confirmed by a larger number of such studies.

However, there are also data that do not confirm any significant superiority of AS with the separate stage of hard dental tissues etching. J. H. Jang et al. compared self-etching systems, where all three stages of adhesive preparation are combined into one, and total etching systems, where the stages of adhesive preparation are differentiated, in two ways: by examining the bursting micro-strength, noting the nature of the burst, and by comparing images of the adhesive layer obtained using a transmission electron microscope. In the first case, the authors noted the advantage of some kinds of total etching systems over self-etching adhesives, but noted that the break line always ran along the border of the adhesive interface. In the second case, there was no significant difference in the structure of the adhesive layer. The authors noted that in general, in terms of the results achieved, both self-etching systems and total etching systems are equally effective [13].

It is possible that statistically more reliable results can be obtained by using more samples of hard tissue of primary teeth and a greater variety of representative products of the selected AS generations.

The results of the study can be used by practicing pediatric dentists making a choice of an adhesive protocol for routine use, as well as by other researchers aiming at a more detailed study of adhesion of restorations to the hard tissues of primary teeth.

Conclusion

4th and 5th generation AS demonstrate high penetration ability, which allows us to recommend their use in the restoration of temporary teeth. The authors attribute the result to the fact that dentin conditioning with 37% orthophosphoric acid allows for more effective removal of the smear layer, enabling deeper penetration of the AS components into the dentine tubules. Thus, the exclusion of conditioning as a separate stage adversely affects the adhesion to the dentin of primary teeth. The authors want to emphasize the convenience and accura-

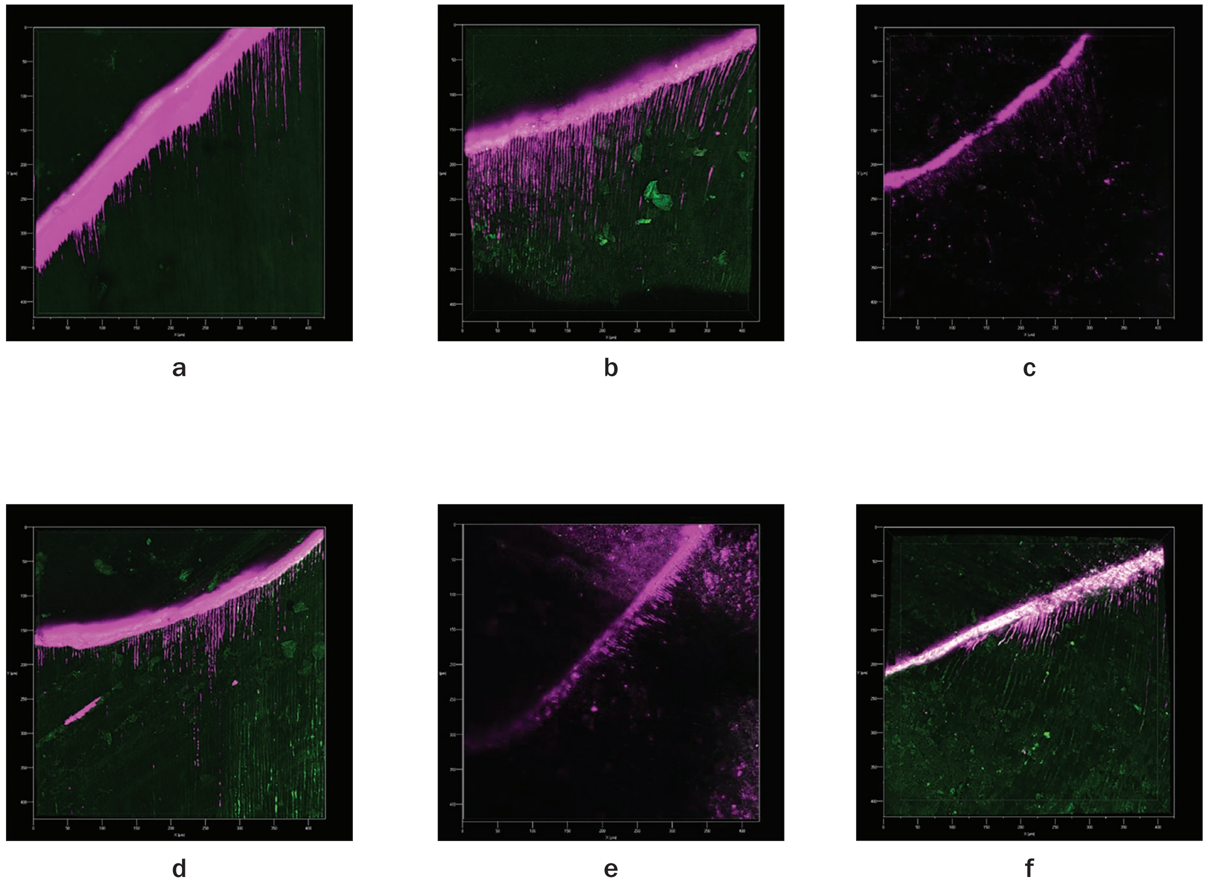

Рис. 2. 3D-реконструкция флуоресцентного изображения спила временного зуба при использовании различных адгезивных систем. Зеленым цветом подсвечены участки автофлуоресценции тканей зуба, розовым цветом – проникающая в дентин адгезивная система, меченная родамином B:

a – Bond A (IV поколение);
b – Bond B (V поколение);
c – Bond C (V поколение);
d – Bond D (VII поколение);
e – универсальная АС Bond E;
f – универсальная АС Bond F

Fig. 2. 3D reconstruction of a fluorescent image of temporary tooth sawn with various adhesive systems. Autofluorescence is shown in green, adhesive system labeled with rhodamine B – in pink:

a – Bond A (generation IV);
d – Bond B (generation V);
c – Bond C (generation V);
d – Bond D (generation VII);
e – universal Bond E;
f – universal Bond F

cy of their research method, fluorescent confocal microscopy, and hope for its further popularization in dentistry. This method can later be used to assess the penetration capacity of endodontic pastes and sealers, as well as to study colonies of microorganisms in the oral cavity and on hard dental tissues.

The performance of work was actively supported by the Core Facilities Center of the General Physics Institute of the RAS "Technological and Diagnostic Center for the Production, Research and Certification of Micro and Nanostructures."

REFERENCES

1. Welbure R.R., Duggal M.S., Hosey M.T. *Paediatric dentistry*. Oxford University Press, 2005. 445 p.
2. Ozer F., Blatz M.B. Self-Etch and Etch-and-Rinse Adhesive Systems in Clinical Dentistry, *Compend Contin Educ Dent*, 2013, vol. 34(1), pp. 12–18.
3. Aslanyan M., Eremin O., Trufanova Yu., Aslanyan Mel.A., Bykova O., Zavyalov A. Use of adhesive systems in dentistry: past and present, *Saratovskii nauchno-meditsinskii zhurnal*, 2018, no. 2, pp. 234–239.
4. Edward J.S. Bonding systems for restorative materials - a comprehensive review. *Pediatric Dentistry*, 1998, vol. 20(2), pp. 80–84.
5. Semikozov O., A clinical review on self-etch adhesives, *Problemy stomatologii*, 2010, vol. 4, pp. 12–14. (in Russ.)
6. Cameron A.C., Widmer R.P. *Handbook of pediatric dentistry*. Oxford, Elsevier Health Sciences, 2013.
7. Sofan E., Sofan A., Palaia G., Tenore G., Romeo U., Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type, *Ann Stomatol (Roma)*, 2017, vol. 8(1), pp. 1–17.
8. Choi A.N., Lee J.H., Son S., Jung K.H., Kwon Y.H., Park J.K. Effect of Dentin Wetness on the Bond Strength of Universal Adhesives. *Materials*, 2017, vol. 10(11), pp. 12–24.
9. Rosa W., Piva E., Silva A. Bond strength of universal adhesives: A systematic review and meta-analysis, *Journal of dentistry*, 2015, vol. 43(7), pp. 765–776.
10. Munoz M.A., Luque I., Hass V., Reis A., Loguercio A.D., Bombarda N.H. Immediate bonding properties of universal adhesives to dentine, *Journal of Dentistry*, 2013, vol. 41, pp. 404–415.
11. Munoz M.A., Sezinando A., Luque-Martinez I., Szesz A.L., Reis A., Loguercio A.D. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives, *Journal of Dentistry*, 2014, vol. 42, pp. 595–602.
12. Lenzi T., Gimenez T., Tedesco T., Mendes F., Rocha R., Raggio D. Adhesive systems for restoring primary teeth: a systematic review and meta-analysis of in vitro studies, *International Journal of Paediatric Dentistry*, 2016, vol. 26(5), pp. 364–375.
13. Jang J.H., Lee M.G., Woo S.U., Lee C.O., Yi J.K., Kim D.S. Comparative study of the dentin bond strength of a new universal adhesive, *Dent Mater*, 2016, vol. 35(4), pp. 606–618.

ЛИТЕРАТУРА

1. Welbure R.R., Duggal M.S., Hosey M.T. Paediatric dentistry. – Oxford University Press, 2005. – 443 p.
2. Ozer F., Blatz M.B. Self-Etch and Etch-and-Rinse Adhesive Systems in Clinical Dentistry // Compend Contin Educ Dent. – 2013. – Vol. 34(1). – P. 12–18.
3. Асланян М.А., Еремин О.В., Труфанова Ю.Ю., Асланян Мел.А., Еремин А.О., Быкова О.А., Завьялов А.И. Применение адгезивных систем в стоматологии: прошлое и настоящее // Саратовский научно-медицинский журнал. – 2018. – № 2. – С. 234–239.
4. Edward J.S. Bonding systems for restorative materials - a comprehensive review // Pediatric Dentistry. – 1998. – Vol. 20(2). – P. 80–84.
5. Семикозов О.В. Клинический взгляд на самопротравливающие адгезивы // Проблемы стоматологии. – 2010. – № 4. – С. 83–87.
6. Cameron A.C., Widmer R.P. Handbook of pediatric dentistry. – Oxford: Elsevier Health Sciences, 2013.
7. Sofan E., Sofan A., Palaia G., Tenore G., Romeo U., Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type // Ann Stomatol (Roma). – 2017. – Vol. 8(1). – P. 1–17.
8. Choi A.N., Lee J.H., Son S., Jung K.H., Kwon Y.H., Park J.K. Effect of Dentin Wetness on the Bond Strength of Universal Adhesives // Materials. – 2017. – Vol. 10(11). – P. 12–24.
9. Rosa W., Piva E., Silva A. Bond strength of universal adhesives: A systematic review and meta-analysis // Journal of dentistry. – 2015. – Vol. 43(7). – P. 765–776.
10. Munoz M.A., Luque I., Hass V., Reis A., Loguercio A.D., Bombarda N.H. Immediate bonding properties of universal adhesives to dentine // Journal of Dentistry. – 2013. – Vol. 41. – P. 404–415.
11. Munoz M.A., Sezinando A., Luque-Martinez I., Szesz A.L., Reis A., Loguercio A.D., Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives // Journal of Dentistry. – 2014. – Vol. 42. – P. 595–602.
12. Lenzi T., Gimenez T., Tedesco T., Mendes F., Rocha R., Raggio D. Adhesive systems for restoring primary teeth: a systematic review and meta-analysis of in vitro studies // International Journal of Paediatric Dentistry. – 2016. – Vol. 26(5). – P. 364–375.
13. Jang J.H., Lee M.G., Woo S.U., Lee C.O., Yi J.K., Kim D.S. Comparative study of the dentin bond strength of a new universal adhesive // Dent Mater. – 2016. – Vol. 35(4). – P. 606–618.