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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

# The effect of propolis solution on root surface treatment in replanted teeth

O efeito da solução de própolis no tratamento da superfície radicular em dentes reimplantados

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## ABSTRACT

**Objective:** The objective of this study was to assess the effect of propolis solution on root surface treatment in cases of delayed tooth replantation following chemical or mechanical removal of the periodontal ligament. **Material and methods:** Maxillary right central incisors of 36 male rats (Wistar) were extracted, kept in a dry environment for 15 minutes and then distributed into six groups, as follows: mechanical-propolis and mechanical-fluoride (mechanical removal followed by immersion in 6% propolis and 2% sodium fluoride, respectively), mechanical (mechanical removal only), chemical-propolis and chemical-fluoride (chemical removal followed byimmersion in 6% propolis and 2% sodium fluoride, respectively), and chemical (chemical removal only). Following periodontal ligament removal, root canals were filled with calcium hydroxide paste and replanted. After 60 days, histological analysis with light microscopy was carried out to detect areas of dentoalveolar ankylosis and root resorption. **Results:** Results revealed that chemical removal of the periodontal ligament was associated with slightly better tissue repair findings when compared with mechanical removal. **Conclusions:** The use of 6% propolis solution encouraged tissue repair, while the 2% sodium fluoride solution combined with chemical removal resulted in a better-organized healing process, with less destruction of dentine when compared with the 6% propolis solution.

## KEYWORDS

Tooth injuries; Tooth ankylosis; Root resorption; Tooth avulsion; Tooth replantation; Propolis.

## RESUMO

**Objective:** O objetivo deste estudo foi avaliar o efeito da solução de própolis no tratamento da superfície radicular em casos de reimplante dentário tardio após a remoção química ou mecânica do ligamento periodontal. **Material e métodos:** incisivos centrais superiores direitos de 36 ratos machos (Wistar) foram extraídos, mantidos em ambiente seco por 15 minutos e, em seguida, distribuídos em seis grupos, como segue:-própolis-mecânico e flúor-mecânico (remoção mecânica seguida por imersão em 6% própolis e 2% de fluoreto de sódio, respectivamente), mecânica (somente remoção mecânica), químico-própolis e químico-flúor (remoção química seguida de imersão em própolis 6% e fluoreto de sódio a 2%, respectivamente), e química (remoção química somente ). Após a remoção do ligamento periodontal, os canais radiculares foram preenchidos com pasta de hidróxido de cálcio e reimplantadas. Após 60 dias, análise histológica com microscopia de luz foi realizado para detectar áreas de anquilose dentoalveolar e reabsorção radicular. **Resultados:** Os resultados revelaram que a remoção química do ligamento periodontal foi associada a efeitos de reparação de tecido ligeiramente melhor quando comparado com a remoção mecânica. **Conclusões:** O uso da solução de própolis 6% estimulou a reparação de tecidos, enquanto a solução de fluoreto de sódio a 2% combinado com a remoção química, resultou em um processo de cura mais bem organizado, com menos destruição da dentina quando comparado com a solução de própolis 6%.

## PALAVRAS-CHAVE

Lesões dentárias; Anquilose dental; Reabsorção radicular; Avulsão dentária; Reimplante dentário; Própolis.

## INTRODUCTION

Much research has been conducted recently on dentoalveolar traumas. Tooth avulsion has one of the least favorable prognoses of all types of dental trauma. At this age, maxillary central incisors are erupting, and the periodontal ligament has a loose structure surrounding the root, offering little resistance to extrusive forces [1,2].

The gold-standard treatment for avulsed teeth is immediate replantation, but this is not always possible; in fact, in the majority of cases, tooth replantation is delayed. This situation compromises prognosis, since the excessive time that the teeth spend in dry, extra-alveolar environments may provoke necrosis of periodontal ligament cells. Therefore, storage of avulsed teeth in an appropriate medium until replantation and the initial approach adopted by the dental surgeon are extremely important for a successful treatment [3-5].

The optimal replantation time for the best prognosis has been declared as 5 minutes. The findings indicate that the risk of resorption increases dramatically after 5 minutes of dryness while the increase after 20 minutes appears to be of a lower order [6].

Studies on delayed replantation have tested several substances not only as storage media for preserving the viability of the periodontal ligament, but particularly as root surface treatments, intended to prevent the occurrence of root resorption and dentoalveolar ankylosis and to help restore the dentoalveolar articulation [6-9].

Propolis is one of the few natural medications that has remained popular over the years [10]. This natural resinous, dark yellow substance [11] has countless properties (including antiinflammatory and antibacterial action), which may vary according to the region and the time of the year when it is collected. Therefore, comparative studies have been conducted to obtain a standardization, i.e., to find an association between chemical composition and biological activity, thus allowing a more precise identification of the active principles and a more effective therapeutic application of propolis [12,13].

Although the use of propolis in dental applications has become increasingly widespread over time [14], very little is known about its effects on the treatment of dentoalveolar traumas. The few studies already carried out have reported promising results [15-18], suggesting that more attention should be devoted to assessing the benefits of the use of propolis in the dental clinical practice.

The aim of this study was to analyze the effects of 6% propolis solution on root surface treatment in delayed replantation of rat teeth, with a focus on its role in eliminating or inhibiting root resorption and dentoalveolar ankylosis.

## MATERIAL AND METHODS

The present study is in accordance with the National Research Council Guidefor the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee at Catholic University Rio Grande of Sul (PUCRS), Brazil.

Thirty-six male Wistar rats, with body weight between 300 and 500 g, were anesthetized with 0.05 ml/100g of ketamine (Ketamin, Agener União, Embu-Guaçu, Brazil) and 0.025 ml/100g of xylazine hydrochloride (Calmiun, Agener União, Embu-Guaçu, Brazil) via intraperitoneal injection. Following intraoral and perioral antisepsis, the maxillary right central incisor of each animal was extracted and its dental papilla removed with a no. 15 scalpel blade (Med Blade, Huaiyin Med, Jiangsu, China). The teeth were fixed by the crown to a sterile bone wax plate (Ethicon, Johnson & Johnson Company, São José dos Campos, Brazil) and left exposed for 15 minutes. Then, the pulp was

removed with a size #15 endodontic file (Flex-R Roane Tip File, Moyco Union Broach, New York, USA). Canals were cleaned with 5 ml of 0.9 % saline solution (Texon® Pharmaceutical industry Ltda, Viamão, Brazil).

Teeth were then distributed into six groups: MP, mechanical removal of the periodontal ligament and tooth immersion in propolis solution; MF, mechanical removal of the periodontal ligament and tooth immersion in sodium fluoride solution; M, mechanical removal of the periodontal ligament, no tooth immersion; CP, chemical removal of the periodontal ligament and tooth immersion in propolis solution; CF, chemical removal of the periodontal ligament and tooth immersion in sodium fluoride solution; C, chemical removal of the periodontal ligament, no tooth immersion.

Mechanical removal of the periodontal ligament was carried out by scraping the entire palatal surface of the root in crown-apex direction using a no. 15 scalpel blade. Chemical removal was performed by immersion of the tooth in 1 % sodium hypochlorite solution (Cloro Rio, Rioquímica, São Caetano do Sul, Brazil) for 30 minutes. Following ligament removal, teeth in the MP and CP groups were immersed in 5 ml of 6 % propolis solution (Lua de Mel Ind. Api. Nat. Ltda., Viamão, Brazil), and those in the MF and CF groups were immersed in 5 ml of 2 % sodium fluoride solution (Vigodent, DFL, Rio de Janeiro, Brazil), at pH 5.5; in both cases, immersion lasted for 20 minutes.

After the treatments, root and internal surfaces were irrigated with 20 ml of 0.9 % saline solution. Root canals were dried with absorbent paper points (Endopoints® Indústria e Comércio Ltda, Paraíba do Sul, Brazil) and filled with calcium hydroxide paste (Calen®, S.S.White, Rio de Janeiro, Brazil). Sockets were carefully probed to check for unorganized coagulation before replantation. Dental retention was not employed [19,20].

Animals were given systemic antibiotic therapy at a single dose of 20.000 units/kg of penicillin G benzathine (Benzetacil, Eurofarma Laboratories Ltda., São Paulo, Brazil) via intramuscular injection, and 100 mg/kg of paracetamol (Merck, Rio de Janeiro, Brazil) via intraperitoneal injection for postoperative pain control. For the first 30 days after replantation, rats were fed with Nuvital® powdered chow (Nuvital Nutrients S/A, Colombo, Brazil), returning to pelleted chow after that. Water was available ad libidum throughout the experiment.

After 60 days, the animals were sacrificed by inhalation of an isoflurane overdose (Isoforine, Cristália, Itapira, Brazil). The hemimaxillae containing the replanted teeth were removed and immersed in 10 % formalin for 24 hours. For the histological analysis, four slides were prepared from each tooth, with 5-$\mu$m-thick longitudinal sections, from the cervical to the apical region, stained with hematoxylin and eosin (HE). A descriptive analysis of the palatal surface of the middle third of the root was carried out using a light microscope (Olympus BX50, São Paulo, Brazil).

## RESULTS

Two animals died during the experiment. The analysis and description of the slides focused on the following aspects: intensity of the inflammatory process, reinsertion of periodontal ligament fibers, presence/absence of replacement resorption; presence/absence of inflammatory resorption; presence/absence of ankylosis; bone tissue characteristics.

Results obtained in each group are described below:

***MP group: mechanical removal of the periodontal ligament and tooth immersion in propolis solution***

This group showed little, unorganized trabecular bone formation. Areas of replacement resorption were observed in all specimens, as

well as areas of resorption filled with connective tissue. Dentinal tissue presented a more linear pattern, and did not show a large number of resorption lacunae. A dense, highly cellular layer of connective tissue was found adjacent to the dentine, with little permeation of bone tissue (Figure 1).

**MF group: mechanical removal of the periodontal ligament and tooth immersion in sodium fluoride solution**

The alveolar bone tissue showed an organized pattern. There were areas of dentinal tissue affected by replacement resorption, which occurred in a more regular and linear pattern along the root axis. There were some areas of resorption filled with fibrous connective tissue. Areas of ankylosis were also observed, with no signs of dentine resorption and no evidence of osteoclast activity. One specimen showed an area of periodontal ligament reinsertion with oblique fibers (Figure 2).

**M group: mechanical removal of the periodontal ligament, no tooth immersion**

This group showed unorganized bone tissue, with the presence of resorption lacunae containing blast cells. Replacement resorption was observed in the dentinal tissue, forming lacunae containing osteoclasts in the majority of specimens. One specimen showed a smaller replacement resorption area, with predominance of ankylosis and absence of dentinal tissue destruction. In one specimen with no dentinal tissue destruction, a layer of dense connective tissue was observed between the tooth and bone along the root axis (Figure 3).

**CP group: chemical removal of the periodontal ligament and tooth immersion in própolis solution**

The majority of the alveolar bone tissue in this group was compact, with rare trabecular areas. Ankylosis was present in some specimens. The dentinal tissue showed areas of replacement resorption with few lacunae and no clast cells. Dense connective tissue was present, with

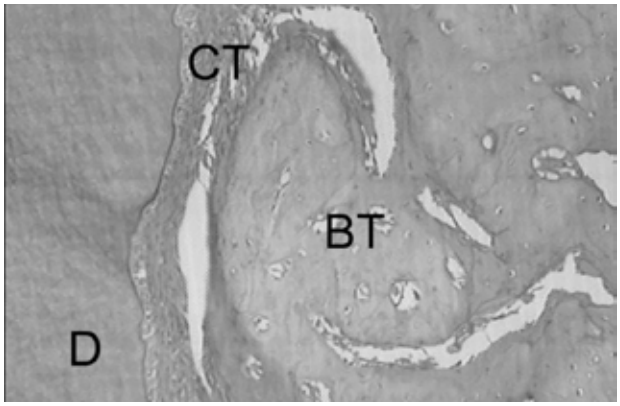


**Figure 1 -** MP Group – Layer of connective tissue (CT) brought between tooth and bone; bone tissue (BT); dentine (D). H.E., original 100x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

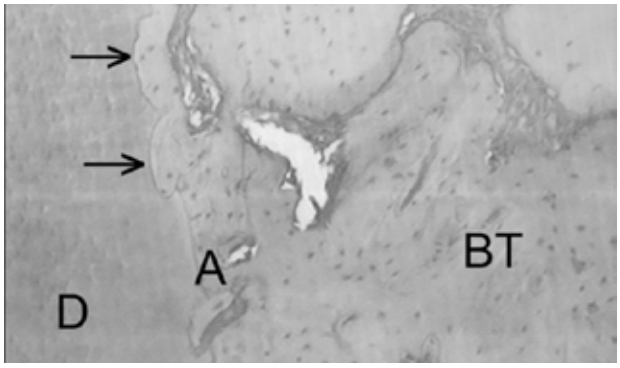


**Figure 2 -** MF Group – areas affected by replacement resorption (arrows); ankylosis (A); dentine (D); bone tissue (TO). H.E., original 200x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

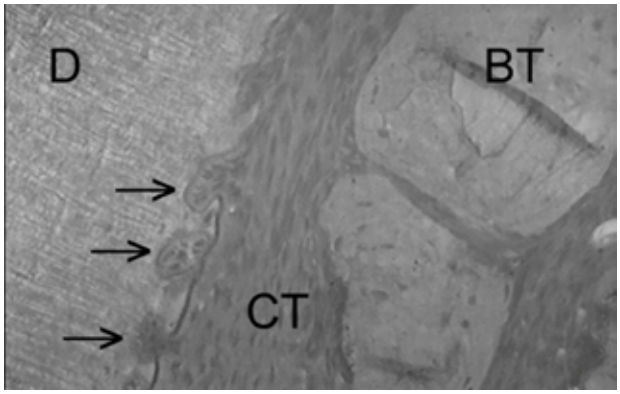


**Figure 3 -** M Group – dense connective tissue (CT) between dentin (D) and bone tissue (BT). Replacement resorption in the dentinal tissue, forming lacunae containing osteoclastos. H.E., original 200x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

unorganized fibers and a moderate chronic inflammatory process (Figure 4).

**CF group: chemical removal of the periodontal ligament and tooth immersion in sodium fluoride solution**

This group showed organized alveolar bone tissue and few areas of replacement resorption in the dentinal tissue. Ankylosis predominated in all sections. There were large amounts of loose connective tissue interposed with the bone tissue, with a small number of chronic inflammatory cells (Figure 5).

**C group: chemical removal of the periodontal ligament, no tooth immersion**

In this group, the alveolar bone tissue was highly cellularized and unorganized, with extensive irregular areas of replacement resorption. One specimen showed less intense resorption and presence of ankylosis with a small number of clast cells. Dense, highly cellular connective tissue was present, intermingled with bone trabeculae. In some areas, the connective tissue was interposed between the bone and tooth, with a small number of lymphocytes (Figure 6).

## DISCUSSION

Sodium fluoride is known to be the substance of choice for root surface treatment in late tooth replantation [1,3,21,22]. According to Andreasen et al. [1], sodium fluoride acts strengthening the root surface structure through the formation of fluorapatite, a substance that is toxic to the cells that resorb hard tissues. Nevertheless, treatment with sodium fluoride is not totally effective because it does not exclude the possibility of root resorption. The fluoride is incorporated into the superficial layer of the root only, i.e., it does not penetrate the deeper layers of the dentine. As a result, whenever the cementum resistance becomes insufficient, there is progression to root resorption in the dentine [23].

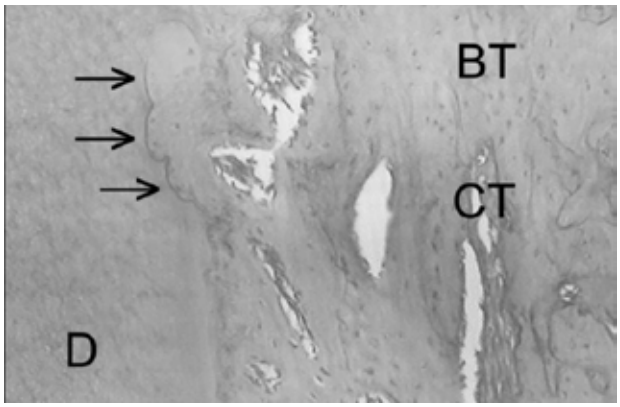


**Figure 4 -** CP Group - replacement resorption (arrows); conective tissue (CT); dentina (D); bone tissue (BT) with rare trabecular areas. H.E., original 100x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

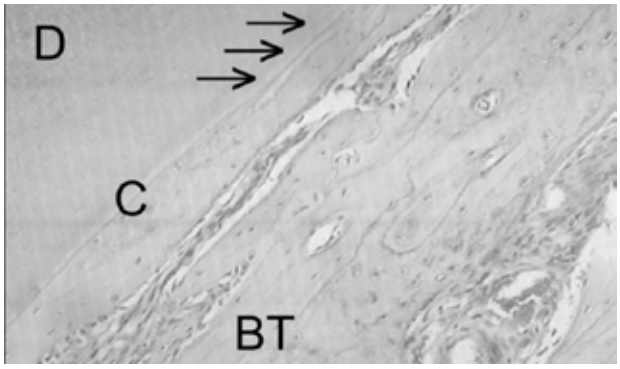


**Figure 5 -** CF Group – ankylosis (arrows); preservation of dentin (D) and cementum (C); bone tissue (BT). H.E., original 100x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

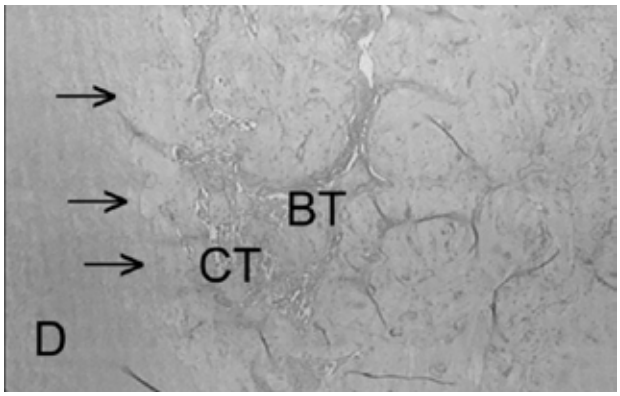


**Figure 6 -** C Group – irregular areas of replacement resorption in dentin (D) (arrows); conective tissue (CT), intermingled with bone tissue (BT). H.E., original 100x.
Source: Data of the research, Postgraduate Program – FO/PUCRS (2008).

In our study, many specimens showed extensive areas of resorption. However, the groups treated with 2% sodium fluoride (MF and CF) showed a more organized healing process when compared with groups M, C, MP, and CP. Furthermore, groups MF and CF showed smaller areas of resorption and more extensive areas of ankylosis, with few areas of dentine destruction.

Much research has been carried out to investigate substances that would offer better conditions for tissue repair after tooth replantation. Some studies have found that propolis produces better effects than Hank's balanced salt solution (HBSS), milk, saliva and saline solution as a storage medium for maintaining the viability of cells in the periodontal ligament [15-18]. However, to date, only a few studies have evaluated the effects of propolis solution used in the root surfasse treatment of late replanted teeth. Gulinelli et al. [17] tested a 15% propolis solution and obtained results that were statistically similar to those associated with 2% sodium fluoride in terms of external root resorption.

There is no consensus on the ideal concentration of propolis to promote the best effects on the periodontal ligament. Our selection of a 6% propolis solution, the lowest concentration available, is accordance with Ozan et al. [16], who studied propolis solution as a storage medium for avulsed teeth and observed that lower concentrations (10%) yielded better results when compared with higher concentrations (20%).

The results of chemical vs. mechanical removal of the periodontal ligament are still under debate. In this study, replantations made after chemical removal were in slightly better condition when compared to the mechanical removal group. This is probably a result of the variable manual pressure employed during scraping with the scalpel blade, which may have resulted in cementum removal, thus exposing dentinal tubules and allowing larger areas of root resorption to develop. Furthermore, mechanical procedures may fail to remove all periodontal ligament cells, with necrotic periodontal ligament remnants adhering to the root surface, which was probably the case of the one MF specimen showing periodontal ligament insertion into the cementum. On the other hand, the chemical action of 1% sodium hypochlorite solution for 20 minutes may have completely removed the periodontal ligament. Finally, another disadvantage of the mechanical method if compared with the chemical one refers to the risks of physical injury to the cementum while using the scalpel blade.

The concentration of 1% in the sodium hypochlorite solution was chosen based on the findings of Sonoda et al. [24], who observed better results in groups treated with concentrations below 3%. Those authors also pointed out that the use of concentrations greater than 5% in rats may cause a more accentuated removal of organic components from the root surface, thereby exposing a greater portion of the mineral component of the cementum and consequently encouraging root resorption.

Root canals were filled using calcium hydroxide paste because condensation of guttapercha cones would risk fracturing the root walls. The same can be observed in several other studies with a similar methodology [15,17,24,25]. In our study, the use of calcium hydroxide paste probably had an influence on the low occurrence of inflammatory resorption, as a result of the high local pH of the material, which reduces osteoclast and alkaline phosphatase activity and thus delays the resorption process [26,27].

In some specimens, only the region adjacent to the buccal surface of the root showed an intense chronic inflammatory process. This can probably be explained by absence of the protection offered by the periodontal ligament in this area, causing the dental enamel to be in direct contact with bone tissue.

In this study, the propolis solution effectively contributed to the tissue repair

process, since in the MP group, areas of replacement resorption were present but at a lesser intensity when compared with group M. This should be seen as a stimulus for the development of further research into the effects of propolis solution on the periodontal ligament, cementum and dentine, so as to better define its properties and allow it to be indicated not only for root surface treatment but also as a storage medium for avulsed teeth. Specifically, studies should focus on the effects of the antimicrobial action of propolis on the oral and periodontal microbiota.

Another factor that has been the subject of debate in the literature is the occurrence of bacterial infections. When infections involve both tooth and socket, they may trigger or accelerate the dental resorption process, with important consequences for the success of replantation. According to Consolaro [27], infection-induced inflammation causes the accumulation, via the inflammatory exudate, of several mediators that induce and stimulate osteoclasis. On the other hand, contamination by anaerobic bacteria is more severe, and prognosis more doubtful. During pre-replicative periods, anaerobic bacteria release large amounts of lipopolysaccharides or endotoxins, promoting intense leukocytic cellular stress and increasing local levels of IL-1 cytokine and tumor necrosis factor (TNF), two powerful amplifiers of the inflammatory and resorption processes. Therefore, Consolaro [27] recommends the use of broad-spectrum antibiotics in replantation cases. In this study, all animals were given systemic antibiotic therapy with penicillin G benzathine, a well-established antibiotic (both in the literature and in the clinical practice), which has been shown to prevent and/or minimize root resorption [3,16,17,24].

The biological properties of propolis are primarily associated with its phenolic constituents. One of such constituents are the flavonoids, plant extracts that inhibit prostaglandins and disinfect tissues [28]. Other elements also found in propolis, such as iron and zinc, are considered extremely important for collagen synthesis [29]. Although it is important to consider that the composition and properties of propolis may vary according to the region where it is collected [14,30], studies carried out in a variety of different regions have shown that the substance has overall favorable effects on tissue repair [15,17].

Finally, the postoperative period of 60 days employed in this study was also used by Gulinelli et al. [17]. Analyses carried out before 60 days would allow observation of inflammatory resorption in specific regions only, while periods greater than 60 days would provide data on the occurrence of ankylosis and the progression of replacement resorption.

## CONCLUSIONS

The 6% propolis solution used in our study as root surface treatment encouraged tissue repair, but did not prevent root resorption or dentoalveolar ankylosis. When combined with chemical removal of the periodontal ligament, the use of 2% sodium fluoride solution was associated with a more organized healing process and less dentine destruction when compared with the 6% propolis solution. Chemical removal with 1% sodium hypochlorite solution was associated with smaller areas of dental resorption filled with connective tissue when compared with mechanical removal of the periodontal ligament.

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Date submitted: 2015 Jun 01

Accept submission: 2015 Sep 21