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Experimental amine-epoxide sealer: a physicochemical study in comparison with AH Plus and EasySeal

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Abstract

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Aim To compare selected physicochemical and biological properties of an experimental sealer with those of two commercially available sealers.

Methodology AH Plus and EasySeal were used as model materials for commercially available amine-epoxide sealers. They were mixed as stated by the manufacturer. The two components of experimental sealer EvoSeal A were mixed 1 : 1 vol%. The setting time was determined in two different ways: first, by setting of sealers in a temperature- and moisture-controlled environment followed by testing with a Gilmore needle and secondly, by oscillating measurements of setting behaviour using a rheometer. Differential scanning calorimetry (DSC) of the sealer was performed for comparison of thermal properties. Flow and film thickness were determined by applying pressures of 100 g and 15.3 kg, respectively, on the materials between two glass plates and measuring the diameters of the compressed sealer and the thickness with a micrometer gauge. Solubility of set materials was conducted by layering the samples with water, storing in a temperature- and humidity-controlled environment and evaporating the solvent. The solved sealer parts were then weighed. The radiopacity was measured in an X-ray experiment comparing radiopacity of a cured sealer to an aluminium step wedge. Volume shrinkage was defined by measuring the densities of samples before and after setting. The film thickness, fluidity, curing time, radiopacity and solubility of the test materials were performed as specified in DIN EN ISO 6876:2010 draft. The volume shrinkage was determined in a method adapted from standard DIN 13907:2007-01. Antibacterial activity was tested against Gram-positive *Streptococcus oralis* cultures in a contact test based on standard ISO 22196:2011 (E). Statistical analysis was performed using Mann–Whitney *U*-test where applicable. Significant differences were determined with $P < 0.05$.

Results The experimental sealer, EvoSeal A, reached standard specifications. In terms of film thickness, the highest value was measured for EvoSeal A with a film thickness of 27 μm, comparing to 6 μm for EasySeal ($P \leq 0.001$) and 8 μm for AH Plus ($P \leq 0.001$). Comparing the flow, all values corresponded to EasySeal with a diameter of 17.3 mm. The only significant difference was determined for AH Plus compared to EvoSeal A ($P = 0.0353$). Volume shrinkage of EvoSeal A was 48% smaller compared to EasySeal and approximately 20% lower compared to AH Plus. The shortest curing time was determined for EvoSeal A (3.0 h) followed by EasySeal (4.1 h) and AH Plus (24 h). For all groups, significant differences were observed ($P \leq 0.001$). EvoSeal A had a significantly higher radiopacity than EasySeal ($P \leq 0.001$) but significantly lower values than AH Plus ($P \leq 0.001$). The solubility of AH Plus and EvoSeal A was <0.5% ($P = 0.2435$). Compared to EasySeal with a solubility of 2.7%, significant differences were observed ($P \leq 0.02$). Three weeks after setting, EasySeal and EvoSeal A still had an antibacterial effect against *S. oralis* in contrast to AH Plus. In this respect, comparing AH Plus with

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EvoSeal A and EasySeal, respectively, significant differences were observed ($P \leq 0.001$). No significant differences between EasySeal with EvoSeal A ($P = 0.540$) were determined.

Conclusions The physical and chemical properties of the experimental sealer EvoSeal A were comparable to the two commercially established sealers EasySeal and AH Plus.

Keywords: AH Plus, antibacterial, dental root canal, EasySeal, EvoSeal, physicochemical.

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Introduction

The handling properties of endodontic sealers and their clinical behaviour may be characterized by laboratory tests on their physical properties as defined in DIN EN ISO 6876 of the International Organization of Standardization (ISO). Although a number of new endodontic sealers, techniques and physicochemical methods have been developed, the ideal root canal sealer remains elusive (Adanir *et al.* 2005, Versiani *et al.* 2006, Resende *et al.* 2009, Schäfer *et al.* 2015). The most established sealers are epoxy resin-based, due to their advantageous physicochemical properties (Chandra *et al.* 2014). AH Plus (Dentsply De Trey, Konstanz, Germany), a well-established epoxy resin-based sealer has better apical sealing, higher radiopacity and higher long-term dimensional stability compared to other endodontic sealers (De Almeida *et al.* 2000, Ørstavik *et al.* 2001, Duarte *et al.* 2010, Marciano *et al.* 2011).

However, significant drawbacks of the present epoxy resin-based sealers include their extended curing time, short- and long-term cytotoxicity and leakage (Zmener *et al.* 1997, De Moor & Hommez 2002, Miletić *et al.* 2005, Sousa *et al.* 2006, Baumgartner *et al.* 2007, Resende *et al.* 2009). These disadvantages foster the need to develop new sealing agents that use nontoxic and accelerating components in combination with permanent antimicrobial properties to decrease secondary inflammation and infection rates.

A two-component experimental sealer, EvoSeal A, was developed at the Heinrich-Heine-University Duesseldorf and awaits approbation of the European Union by CE marking. It comprises a lysine-based amino component, bisphenol-A-diglycidyl ether (BADGE) as the epoxy component, polymerizable quaternary ammonium salts and filling materials (Ritter *et al.* 2013). Recently, the epoxy-amine-based sealer EasySeal (Gebr. Brasseler, Lemgo, Germany) has become commercially available in a two-compartment syringe.

The aim of the present laboratory study is to evaluate the quality of experimental sealer EvoSeal A in comparison with a recently released sealer (EasySeal) and a well-established sealer (AH Plus) in selected physical, chemical and biological properties. The null hypothesis is that EvoSeal A, comprising a novel amino component and polymerizable quaternary ammonium salts as well as filling materials, has inferior physicochemical and biological properties in comparison with EasySeal and AH Plus.

Materials and methods

The root filling materials used in this study were two commercially available epoxy resin-based sealers AH Plus (Dentsply De Trey, Konstanz, Germany) and EasySeal (Komet Dental - Gebr. Brasseler, Lemgo, Germany) and an experimental sealer EvoSeal A (Heinrich-Heine-University Duesseldorf, Germany) (Table 1). All sealers were mixed according to the manufacturer's instructions. Each individual parameter was tested and analysed by a single operator. Setting time, flow, film thickness, solubility and radiopacity were measured according to the specifications given in ISO/DIS 6876:2010. Volume shrinkage was tested using the modified DIN 13907:2007-01 which focuses on light curing polymers only. Antibacterial activity of the three sealers was investigated in a first approximation based on ISO 22196:2011 which describes the testing of antibacterial activity of polymeric surfaces. Also, oscillatory rheology and differential scanning calorimetry were measured to confirm curing times and to compare thermal properties of the three sealers, respectively.

Setting time

A block of aluminium (60 × 140 × 12 mm) was kept for 1 h in a climate chamber with 95% relative humidity (RH) and a temperature of 37 °C. Three ring moulds, having an internal diameter of 10 mm and a thickness of 2 mm, were filled with the sealer

Table 1 Ingredients of the investigated sealers

Sealer	Manufacturer	Ingredients
AH Plus	Dentsply De Trey	Bisphenol-A diglycidyl ether, bisphenol-F diglycidyl ether, calcium tungstate, zirconium oxide, iron oxide, adamantane amine, dibenzyl diamine, tricyclodecane diamine, highly dispersed silicon dioxide, silicon oil
EasySeal	Komet Dental	Diethylenetriamine, Amine-epoxy-based, no further information
EvoSeal A – Experimental Sealer	Heinrich-Heine-University Duesseldorf	Bisphenol-A diglycidyl ether, α- amino-ε-caprolactam, quaternary ammonium salt, gadolinium oxide, barium titanate, diamines, additives

on the prepared block of aluminium. Approximately 2 min after mixing, the moulds were transferred back to the climate chamber. Shortly before the curing time specified by the manufacturer ended, a Gilmore-type needle with a mass of 100 g having a flat end of 2.0 mm in diameter was lowered vertically onto the horizontal surface of each sample. The needle tip was cleaned, and the process was repeated until no indentations were visible. The time that a given material needed to reach this state was noted. The measurement was performed three times for each sealer.

Rheological measurements

Oscillating rheological measurements were performed using the parameter $\tau = 5$ Pa and $f = 1$ Hz on a Haake Mars II rheometer by ThermoFisher Scientific. For this purpose, a plate–plate construction was used. The temperature was set to 37 °C and determined in the measuring plate with an accuracy of ±0.1 °C. Storage modulus G' and loss modulus G'' were measured against time, and the intersection of both curves was assigned to sol-/gel-transition. By definition, before sol-/gel-transition, a material shows properties of a fluid, afterwards properties of a solid. After a certain time, G' and G'' curves reach a plateau, which is a sign for no further reaction. Sol–gel transmission was obtained by the first intersection of G' and G''.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were performed using a Mettler Toledo DSC 822 controller apparatus in a temperature range between −30 and 120 °C with a heating rate of 15 °C min^{-1}. The glass transition temperature (T_g) values were taken from the inflection point of the second heating curves.

Flow

Using a syringe, a volume of 0.5 mL of sealer was placed centrically on a glass slide with the dimensions of 40 × 40 mm, 5 mm width and a mass of 19 g. Three minutes after mixing, a glass plate of same dimensions and weight, and a weight of 100 g were applied centrally on top of the first glass plate. Ten minutes after mixing, the weight of 100 g was removed, and the average of the major and minor diameters of the compressed sealer disc was measured. In case of a deviance of the diameters by 1 mm, the test was repeated. Three measurements were performed for each sealer.

Film thickness

The amine- and the epoxide-paste of each sealer were mixed, and 0.5 g of the material was applied onto a glass plate. A second glass plate was placed vertically over the first one, and the assembly was loaded with a weight of 15.3 kg (150 N). After 10 min, the thickness of the assembly was measured using a micrometer gauge, and the film thickness was determined. The measurement was repeated six times for each sealer.

Solubility

A Teflon® mould measuring 20 mm in inner diameter and 1.5 mm in height was filled with freshly mixed sealer and placed on a glass plate. Another glass plate covered with a sheet was put on the top of the mould, to achieve a smooth surface. This assembly was placed in a climate chamber (95% RH/37 °C) for a period of 1.5 times of the specified setting time. As soon as the samples were removed from the mould, they were weighed to an accuracy of 0.001 g. In each case, the samples were placed two by two inside a crystallizing dish (A). The

samples were covered with 50 mL of distilled water, taking care to avoid any contact between the samples and the inner border of the dish, and were placed into a climate chamber (37 °C, 95% RH) for 24 h. Afterwards, the solution was filtered into a tared crystallizing dish (B). Dish A was rinsed three times with 50 mL of water, which was also filtered into dish B. Dish B was then placed into an oven (110 °C) until the water evaporated, and a constant mass was reached. Before weighing, the dish was cooled to room temperature in a vacuum desiccator, and the experiment was repeated two times for each sealer. Using the determined mass difference between the original and the final mass of dish B, the dissolved portions of the sealer materials could be determined.

Radiopacity

Three ring moulds measuring 1 mm in depth and 10 mm in diameter were placed on a slide. The sealer material was filled in the ring form and covered by a plastic sheet. In order to avoid the formation of bubbles, the sealer was introduced into the moulds using a syringe. The samples were radiographed on occlusal films (*Agfa Dentus M2*®, Heraeus Kulzer, South Bend, IN, USA) containing an aluminium stepwedge 20 mm in width and 50 mm in length and in uniform steps of 0.5 mm (at 10 microns measured accurately). Radiographic images were obtained with exposures set at 70 kV, 0.32 s and a focus-film distance of 30 cm. The optical density was measured using an optical density meter (Densitometer®Type DK-9999-0582-3, 3M Deutschland, Neuss, Germany) and was determined by comparison of the sample and the aluminium wedge in a given X-ray image. The measurement was performed three times for each sealer.

Volume shrinkage based on DIN 13907:2007-01

For the preparation of the test samples, a quantity of 0.3–0.6 g freshly mixed sealer was placed into small plastic bags, which were hermetically sealed afterwards. Five samples were prepared for each sealer. The densities of those samples were determined at intervals of 30 min, 60 min, 120 min, 180 min, 24 h and 1 week. The measurements were performed using a density determination kit (Sartorius, Goettingen, Germany). Between the measurements, the samples were transferred to an incubator (37 °C, 95% RH). The density of the samples was calculated using formula (I).

$$\rho = \frac{W(a) \cdot (\rho(fl) - 0.0012\ g/cm^3)}{G \cdot 0.99983} + 0.0012\ g/cm^3 \quad (I)$$

$G = W(a) - W(fl)$

$W(a)$ = weight of sample in air

$W(fl)$ = weight of sample in water

$\rho(fl)$ = specific density of water depending on temperature

Antibacterial activity based on ISO 22196:2011

The antibacterial activity of the sealer materials 3 weeks after curing was evaluated against *Streptococcus oralis* (DSM 20627, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Bacteria were grown from frozen stock cultures, thawed at 37 °C in water for 3 min and subsequently kept in a TSB broth (Tryptic Soy Broth) in an incubator (Hera Cell®, Heraeus, Hanau, Germany) at 37 °C and 5% CO_2 feed for 24 h. Cells were harvested and resuspended in fresh TSB broth. Bacterial numbers were standardized to an optical density (OD) at 1 ± 0.1 with the aid of a photometer (Bio-RAD SmartSpecTM Plus Spectrophotometer®, Bio-Rad Laboratories, Munich, Germany). Freshly mixed sealer was poured in a metal ring of 10.5 mm in inner diameter and cured in a climate chamber with a constant temperature of 37 °C and a humidity of 99% 3 weeks before the test. Then, 0.2 mL of bacterial suspension was placed on the test material and covered with a cover slip to avoid a desiccation of the bacteria during the incubation period of 24 h. To create a humid atmosphere, the samples were placed in a large petri dish with 17 mL of sterile water, without direct contact between water and the samples. This was followed by incubation for 24 h (37 °C and 5% CO_2 feed). After incubation of the samples, they were rinsed with 700 μL, the cover glass with 200 μL and the small petri dish with 900 μL broth, before 100 μL of the suspension was distributed on an agar plate. After 24 h of storage in an incubator (37 °C and 5% CO_2 feed), the resulting colonies on the agar plates were counted. The experiment was performed for each tested material twenty times. Additionally, a positive control with *Streptococcus oralis* and a negative control with pure TSB broth (Tryptic Soy Broth) were tested.

Statistical analysis

For statistical analysis of the median values in case of setting time, film thickness, radiopacity, solubility and antimicrobial investigations, two groups were statistically compared using the standard Mann–Whitney *U*-test (GraphPad Prism 5, GraphPad Software inc., La Jolla, CA, USA). The range of significant difference of medians was set to $P < 0.05$.

Results

Setting time

ISO/DIS 6876:2010 specifies that the curing time of a sealer must not be more than 10% of those stated by the manufacturers. The curing time provided by the manufacturers of AH Plus is at least 8 h and of EasySeal 24 h, respectively. The lowest curing time was determined for EvoSeal A (3.0 h), followed by EasySeal (4.1 h) and AH Plus (24 h). It should be noticed that the EasySeal sample had elastic properties after 4.1 h, which caused indentation to disappear directly after the Gilmore needle was released (Table 2). For all sample groups, significant differences were observed ($P \leq 0.001$).

Rheology

Gel points, as determined by the intersection of G' and G'', were observed after 112 min (EvoSeal A) and 599 min (AH Plus). For EasySeal, no gel point was obtained, but curves of G' and G'' proceeded horizontally after approximately 250 min.

Glass transition temperatures

The following glass transition temperatures (T_g) of cured sealer materials were measured: AH Plus: 32 °C, EvoSeal A: 64 °C and EasySeal: no glass transition was detected in the scanning region of −30 °C to 120 °C.

Flow

Testing of the flow ability of a root canal sealer according to ISO/DIS 6876:2010 must result in minimum diameters of 17 mm. All tested sealers exhibited similar flow characteristics, as the following diameters were obtained: EvoSeal A (17.0 mm) < EasySeal (17.3 mm) < AH Plus (18.0 mm). No significant differences between AH Plus and EasySeal ($P = 0.374$) and EasySeal and EvoSeal A ($P = 0.397$) were observed. Statistical analysis revealed significant difference between EvoSeal A and AH Plus ($P = 0.0353$).

Film thickness

ISO/DIS 6876:2010 specifies a film thickness for root canal sealers of no more than 50 μm. The highest film thickness measured was EvoSeal A (27 μm) and the lowest EasySeal (6 μm). The thickness of AH Plus was determined as 8 μm. Statistical analysis revealed significant difference between EvoSeal A and AH Plus ($P = 0.0036$) and EvoSeal A and EasySeal ($P = 0.0043$).

Radiopacity

AH Plus and EvoSeal A, but not EasySeal, met the requirements of ISO/DIS 6876:2010 by exhibiting a radiopacity higher than those of 3 mm of aluminium. The radiopacities in increasing order were (optical density Sealer/optical density 3 mm Al): EasySeal (0.96/0.90) < EvoSeal A (0.72/0.86) < AH Plus (0.40/0.95). Statistical analysis demonstrated that EvoSeal A significantly had a higher radiopacity than

Table 2 Physiochemical properties of the investigated sealers

	EvoSeal A	EasySeal	AH Plus
Setting Time [min][a]	180	246	1440
Setting Time (rheology) [min]	112	approx. 250	599
Glass Transition Temperature [°C]	64	–	32
Flow [mm][a]	17.0 ± 1.0	17.3 ± 0.8	18.0 ± 1.0
Film Thickness [μm][a]	27 ± 6	6 ± 2	8 ± 1
Radiopacity [$OD_{1\ mm\ Sealer}/OD_{3\ mm\ Al}$][a]	0.72/0.86 ± 0.02	0.96/0.90 ± 0.03	0.40/0.75 ± 0.03
Solubility [%][a]	0.3 ± 0.1	2.7 ± 0.3	0.1 ± < 0.1
Volume Shrinkage [%][b]	1.8 ± 2.1	3.4 ± 1.4	2.2 ± 2.1

[a]According to ISO/DIS 6876:2010.
[b]According to ISO/DIS 13907:2007-01.

EasySeal ($P = 0.0109$) but lower than AH Plus ($P = 0.0117$).

Solubility

ISO/DIS 6876:2010 states that no more than 3 wt % of a cured root canal sealer should dissolve in water. The solubility of all tested sealers was below this value, although EasySeal exhibited a higher solubility than AH Plus and EvoSeal A: AH Plus (0.1%) < EvoSeal A (0.3%) < EasySeal (2.7%). Statistical analysis demonstrated that EasySeal had a significantly higher solubility than EvoSeal A ($P = 0.0188$) and AH Plus ($P = 0.0161$). Nevertheless, no significant differences in solubility were observed for AH Plus and EvoSeal A ($P = 0.2435$).

Volume shrinkage

The volume shrinkage of the sealers after 1 week of curing was as followed: EvoSeal A (1.8%) < AH Plus (2.2%) < EasySeal (3.4%). Significant differences were not determined because of flaws in the method (see Discussion).

Microbiological investigation

The microbiological investigation after 3 weeks of curing showed the most significant antibacterial properties against *Streptococcus oralis* for EasySeal (median value of 0 colonies, 0% of positive control sample), followed by EvoSeal A (median value of 17 colonies, 1.6% of positive control sample). AH Plus had no antibacterial effect on the test organism. The statistical analysis demonstrated differences between the experimental groups ($P \leq 0.001$).

Discussion

The difference in choice and ratios of amine components and radio-opaque salts of the epoxide-amine-sealer prototype EvoSeal A and the commercially available sealers AH Plus and EasySeal led to different physical and biological properties of the cured resins. The experimental sealer, EvoSeal A, includes the novel bio-based amine α-amino-ε-caprolactam (ACL), which is synthesized from natural nontoxic amino acid L-Lysine (Figure S1, Supporting Information), and a polymerizable quaternary ammonium compound, which is incorporated to ensure antibacterial properties even after setting of the sealer (Mondrzyk *et al.* 2014).

Physical properties of a material give important information about its applicability. Therefore, setting time, flow, film thickness, solubility and radiopacity were measured for each sealer with respect to DIN ISO/DIS 6876:2010. For further analysis, viscosity, behaviour, glass transition temperatures and volume shrinkage were characterized, and a study of the antibacterial properties of the sealers was conducted.

The setting time of the sealers was determined by Gilmore needle indentation tests. The lowest setting time was obtained for EvoSeal A (3.0 h), followed by EasySeal (4.1 h) and AH Plus (24 h). Thus, in contrast to AH Plus, the setting time of EasySeal is in agreement with DIN ISO/DIS standard. However, setting times for AH Plus range usually between 8 and 12 h (Resende *et al.* 2009, Flores *et al.* 2011, Marciano *et al.* 2011, Zhou *et al.* 2013). EasySeal showed elastic properties which made it difficult to obtain exact values for the setting time with a Gilmore needle.

For validation of these results, setting time and viscosity were investigated by oscillatory rheology. As shown in Fig. 1, EvoSeal A reaches sol-/gel-transition (storage modulus G' equal to loss modulus G'') more rapidly by a factor of approximately six in comparison with AH Plus (112 min to 599 h, Figure S3a,c). Shortly after this characteristic point, the moduli reach a plateau at such the sealers can be considered cured. The observations can be affirmed by comparison of complex viscosity η* of the various sealer materials (Figure S3d). Whilst η* rises slowly for AH Plus, it takes on a much steeper curve for EvoSeal A. The setting time of AH Plus obtained by oscillatory rheology corresponds to setting times in literature obtained by Gilmore needle (Schäfer *et al.* 2015). The curves of G' and G'' of EasySeal (Figure S3b) show no sol-/gel-transition in the observed timeframe. As G' is initially already higher than G'' and both moduli rise before proceeding almost parallel, it can be assumed that freshly mixed EasySeal is a flexible material that is only flowable by applying a force. In accordance with these findings, the complex viscosity curve of EasySeal is high to start and reaches a plateau after approximately 250 min. The material is then considered cured.

The glass transition temperature T_g is an often used indicator for flexibility of a given polymer. Hence, the sealers were analysed by DSC. Due to its amorphous features, the T_g of EasySeal lies outside of the

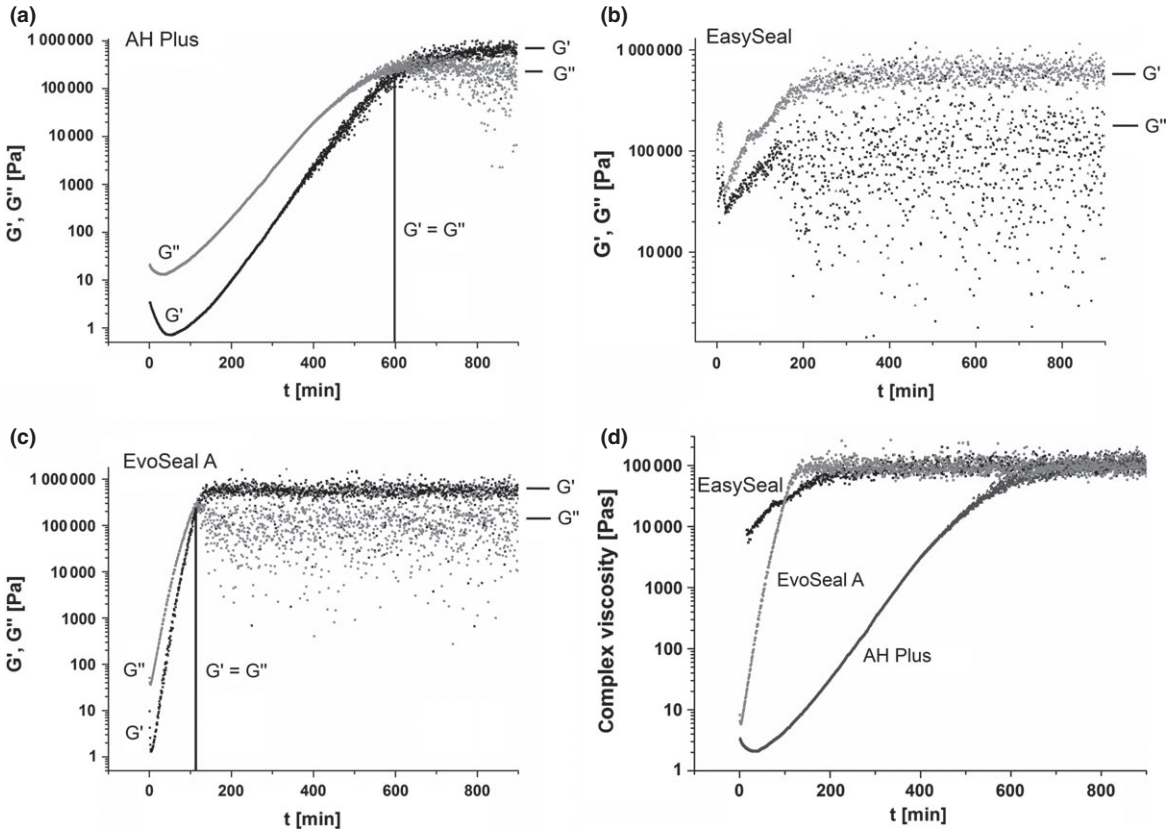

Figure 1 Rheological oscillation measurements. Diagrams of G' and G'' in [Pa] against time in [min] of (a) AH Plus, (b) EasySeal, (c) EvoSeal A and (d) complex viscosity in [Pa·s] against time in [min] of all tested sealers.

scanning range of −20 °C to 120 °C and is further assumed to lie below the lower limit. Taking these results into account, EasySeal can be considered as polymerized but highly elastic material at 37 °C. The measured T_g of sealer prototype EvoSeal A is ca. 30 °C above normal core body temperature, whilst AH Plus has a T_g of ca. 5 °C below normal core body temperature. Therefore, in cured EvoSeal A, inelastic properties predominate in root canals of living patients, whilst in cured AH Plus, elastic properties prevail. Theoretically, a well-rounded sealer is solid enough to seal the root canal but also flexible enough to absorb minor shocks without formation of microfissures in the material, which allow bacteria to pass to the periodontal tissue.

Flow and film thickness are important factors determining sealer topology. For example, once film thickness of a sealer or the particle size is larger than the gap between dentine and Gutta-percha, it can cause an unfavourable displacement of the sealer, which also adversely affects the firmness of the root filling (Wu *et al.* 1997). Whilst a certain flow is necessary for satisfactory distribution of the sealer into anatomical irregularities, an excessive flow rate increases the probability of extrusion into periodontal tissues (Ørstavik 1988). In the literature, a standardized flow between approximately 21–39 mm and a standardized film thickness between 16–44 μm was reported for AH Plus (Resende *et al.* 2009, Marciano *et al.* 2011, Marín-Bauza *et al.* 2012, Zhou *et al.* 2013). The flow (18.0 mm) and film thickness (8 μm) of AH Plus determined in this study were lower. However, the results for AH Plus, EasySeal (17.3 mm 6 μm^{-1}) and EvoSeal A (17.0 mm 27 μm^{-1}) all correspond to standard specifications of a flow of 17 mm diameter and a film thickness of no more than 50 μm, respectively.

Sealers can be exposed to tissue fluids in the root canal and periapical tissues, which can lead to partial dissolution of the sealer material. Thus, the vital

tissue reabsorbs the soluble fractions, and inflammation may occur (Heithersay 1975). AH Plus had the lowest solubility (0.1%), which is in agreement with the values found in literature (Schäfer *et al.* 2015, Zhou *et al.* 2013). Slightly more soluble residues were found for EvoSeal A (0.3%). EasySeal (2.7%) had the highest solubility which exceeds EvoSeal A by a factor of approximately nine.

EvoSeal A had a significantly higher radiopacity than EasySeal but was lower than AH Plus. In the present study, the experimental sealer EvoSeal A met standard specifications relating to X-ray visibility. Hence, the sealer can be clearly distinguished from dentine on a radiograph.

The amount of sealer in a root canal with its inevitable shrinkage should be kept low to prevent porosities and microfissures (Facer & Walton 2003). The volume shrinkage 1 week after application had the lowest values for EvoSeal A (1.8%), followed by AH Plus (2.2%) and EasySeal (3.4%). Nevertheless, the standard deviations, as seen in Table 2, are relatively high, suggesting inconsistencies of the measurement. The method itself is error-prone, as air bubbles can be present in the freshly mixed sealer materials, thus changing their density.

Endodontic sealers comprising quaternary ammonium compounds (quats) revealed antibiofilm effects in recent studies (Bailón-Sánchez *et al.* 2014, Barros *et al.* 2014). In contrast to the sealers tested in these studies, EvoSeal A includes a Quat which is covalently attached to the cured resins, thereby reducing the risk of adverse material effects or allergic reaction as a result of the release of antibacterial substances (Weng *et al.* 2010). The effectiveness of those covalently attached quats against Gram-negative and Gram-positive bacteria was shown recently (Mondrzyk *et al.* 2014). The *in vitro* antibacterial properties of EvoSeal A in comparison with AH Plus and EasySeal after 3 weeks of curing were tested on the bacterial strain *Streptococcus oralis* because they are naturally occurring in the oral microbiota and dental plaque of humans and are potential pathogens (Do *et al.* 2009). A contact test was conducted in respect to ISO 22196:2011, because it is an established way to show antibacterial efficacy of plastics. EvoSeal A comprises a polymerizable antibacterial agent, which kills bacteria only on the surface of the cured sealer material. Hence, a small layer of bacteria should be established on a defined area of the sealer as the sample area is limited by the diameter of the cover slip. However, assumptions, for example on the uniformity of the presumably porous surface, had to be made. As a countermeasure in some degree, the sealers were filled carefully into the ring forms to prevent inclusions of air, thus forming visibly smooth surfaces when cured. Further, the test was planned for a 'mid-term' of 3 weeks, to include evidence of short-term effect as well as a prospect for long-term activity. In conclusion, the method should be seen as a first attempt at evaluating antimicrobial properties.

AH Plus had little effect on Gram-positive *S. oralis* 3 weeks after setting. Also in literature, no relevant antibacterial effect against Gram-positive *Enterococcus faecalis* after setting of the material was reported (Pizzo *et al.* 2006, Slutzky-Goldberg *et al.* 2008). EasySeal and EvoSeal A exhibited an obvious antibacterial effect on *S. oralis* 3 weeks after setting, whereby the antibacterial efficacy was considerably higher with EasySeal. An explanation for the higher antibacterial efficacy of EasySeal is its higher solubility. This leads to a distribution of its constituents, which can potentially act as antibacterial agents, throughout the bacterial medium. On the other hand, only the surface of the cured EvoSeal A showed an antibacterial effect, which resulted in a minor antibacterial effect.

Conclusion

EvoSeal A was comparable to the two commercially available sealers EasySeal and AH Plus. The experimental sealer, EvoSeal A, had positive results as far as curing time, volume shrinkage and antibacterial activity are concerned. Nevertheless, improvements in its radiopacity and further studies regarding its sealing ability (e.g. adhesion to dentine or leakage of the sealer) are necessary. Furthermore, elaborate investigation of its antibacterial efficacy against mature biofilms is necessary.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Synthesis of α-amino-ε-caprolactam from L-lysine-monohydrochloride.

Figure S2. Reaction scheme of the curing of bisphenol-A diglycidyl ether with α-amino-ε-caprolactam.

Figure S3. AH Plus before and after curing at 37 °C for 24 h. The epoxide band at 915 cm^{-1} disappeared nearly completely, whereas the hydroxyl band increased on curing.

Figure S4. EasySeal before and after curing at 37 °C for 24 h. The epoxide band at 915 cm^{-1} disappeared nearly completely. Probably due to hydrogen bonds forming ingredients of EasySeal a broad hydroxyl bands was visible also before curing.

Figure S5. EvoSeal A before and after curing at 37 °C for 24 h. The epoxide band at 915 cm^{-1} disappeared nearly completely, whereas the hydroxyl band increased on curing.