Biofilms on polymeric materials for the fabrication of removable dentures

**Introduction:** Oral microorganisms can contribute to the pathogenesis of many diseases in the oral cavity such as caries, periodontitis, peri-implantitis and denture-related stomatitis. Yet, oral microorganisms may also have a considerable influence on the onset of systemic medical conditions such as lung or cardiovascular diseases. Microorganisms are organized in biofilms and they colonize teeth, mucosa, and dental restorations; the extent to which biofilms are accessible during self-performed oral hygiene varies widely.

**Discussion:** The current demographic trends show that the population is getting older and that an increasing number of elderly and multimorbid patients require nursing care, most of whom already have and/or will receive removable dentures in the future. Impaired motor skills and cognitive abilities often lead to difficulties in self-performed oral hygiene, thus making these patients reliant on others for assistance. The regular accumulation of biofilm on removable dentures, which is not sufficiently removed, may trigger and foster the onset of oral and systemic diseases in immunologically compromised patients. Usually, removable dentures are fabricated from polymeric materials and polymethylmethacrylate is the most frequently used material. In spite of this, many new materials are currently being introduced on the market which can be used to make removable dentures. The range of available materials has become increasingly broad and it includes materials based on polymethylmethacrylate as well as composite-based materials and polymeric materials with a distinct polymer chemistry. Relevant differences exist between the bioadhesion of materials that are processed using classical methods as compared to CAD/CAM-manufacturing.

**Conclusion:** In this context, the current article aims to describe the importance of biofilms on removable dentures, to outline relevant interactions of oral microorganisms with the surface of polymeric materials, and to present strategies for minimizing bioadhesion on removable dentures.

**Keywords:** polymeric materials; removable dentures; microorganisms; biofilms; CAD/CAM-manufacturing
1. The etiology and pathogenesis of biofilm-associated diseases in patients with removable dentures

1.1 The importance of removable dentures

Over the past few decades, both dental care and oral health awareness have notably improved in Germany so that an increasing number of people still have their natural teeth, even at an advanced age [47]. To illustrate this, the number of edentulous patients have halved over the last 20 years; while in 1997 about 25 % of younger seniors between the ages of 65 and 74 years were edentulous, only about 12 % are edentulous nowadays [47]. Nevertheless, almost half of the younger seniors (46 %) wear removable dentures, which underlines their lasting importance in dentistry. For older seniors falling into the age group between 75 to 100 years, and who are also in need of nursing care, the proportion of denture wearers increases up to 86 % [47]. Removable dentures cover large areas of mucous membrane, and thus, provide an extended attachment surface with optimal living conditions for microorganisms; this favors their growth and proliferation together with biofilm formation. Just like for teeth, biofilms that adhere to dentures should be regularly removed. Yet, for older patients in need of nursing care, this is especially difficult to accomplish due to their often limited motor and cognitive abilities (see Fig. 1 and 2).

Nearly 30 % of older seniors receiving nursing care claim that they depend on extra assistance for denture and oral hygiene [47]; this emphasizes the importance of instructing nursing care personnel as well as any other caregivers [93]. Regardless of this fact, the time that caregivers have to help seniors with their daily oral and denture hygiene is limited for a number of reasons [23, 48, 78, 79, 107]. One such motive is that nursing staff have high general care workloads, which means that they have very short time frames for assisting patients with oral hygiene. Secondly, it appears that nurses have deficits with regard to dental training, which leads to difficulties in the recognition, insertion, removal, and cleaning of dentures. Studies have also revealed that care receivers’ refusal to accept help with oral hygiene is a further problem, as is the fear of contact on the part of the caregiver [7]. In spite of these background challenges, the mechanical cleaning of removable dentures is still the gold standard, as the simple application of chemical cleaners is not always sufficient, and should therefore be viewed as a supportive measure, particularly with regard to the removal of microorganisms [32].

1.2 Materials used to produce removable dentures

For the fabrication of removable dentures, materials are differentiated based on whether they are processed into rigid or flexible dentures. Various polymer systems for the fabrication of removable dentures are available on the market, which can be grouped according to the method of processing [90] (see Table 1). The first group consists of materials that can be cured with the help of pressure, heat (special form: microwaves) or light. A second group of materials includes thermoplastic materials, which do not require curing, but are formed by using heat before they solidify. The third group includes industrially cured or thermoplastically processed materials that are subsequently available as CAD/CAM blocks, from which, dental restorations and dentures can be milled.

Polymethylmethacrylate (PMMA) represents the most important self and warm curing resin. It is the most commonly used denture material in everyday practice. PMMA is appealing due to its low cost as well as its ease of repairation and handling [80]. However, the high rigidity of the material has disadvantages such as increased fracture susceptibility and reduced wearing comfort.

Urethane dimethacrylates belong to the group of light-curing resins that are kneadable during processing before their subsequent curing in special ovens with the aid of light. In the fabrication of partial and complete dentures, this processing technique spares the wax-up step [90]. Other applications of light-curing resins include the manufacturing of individual trays, denture relining or orthodontic appliances. In the finished state, they show increased strength compared to warm curing resins [17], but are more brittle and difficult to repair [92].

Figures 1 and 2 Maxillary and mandibular dentures with extensive biofilm deposits and discoloration due to poor denture hygiene belonging to two patients (91 and 77 years old) in need of care
The group of thermoplastics represents plastic materials which are shaped by the application of heat during the manufacturing of removable dentures, and which have flexible properties after cooling. Due to the elimination of the curing process, neither bite lock nor the presence of residual monomers occur [90]. This material group is thus the favored one for use in patients with methyl methacrylate allergies. Important thermoplastic materials are polyamide-based plastics, for example, which can be used to manufacture flexible dentures. These have the advantage of being easier to fit in the mouth in patients with limited mouth opening (Microstomia). Moreover, they have minimal fracture susceptibility due to their high elasticity [90]. In addition, these materials also have esthetic advantages, as gingiva-colored clasp components can be produced from the material. Disadvantages of polyamides are their limited capacity to be repaired and polished [92]. Moreover, due to their elasticity, there is discussion regarding unfavorable pressure distribution, which can result in increased atrophy of the alveolar ridge [11]. Industrially cured thermoplastic PMMA materials can also be classified in the group of thermoplastics. Yet, in contrast to their counterparts, which are manufactured using the conservative process, they have a lower residual monomer content, but at the same time, also a reduced repair capacity.

Another member of thermoplastic materials is polyoxymethylene (POM) which can be used to produce tooth-colored denture frameworks and clasps. Due to the possibility of designing POM frameworks in gingiva color, the fabrication of complete denture bases from POM is conceivable. However, this material cannot be extended and it requires greater spatial dimensions compared to metal clasps and frameworks [92].

Newer processing methods that employ CAD/CAM-manufacturing enable the milling of denture bases, complete dentures and denture frameworks from industrially prefabricated blocks. Industrially pre-cured PMMA is suitable for the production of denture bases or complete dentures. The absence of polymerization shrinkage and a low residual monomer content are significant advantages compared to conservatively processed PMMA. Moreover, the more homogeneous and pore-free nature of CAD/CAM materials appears to have a positive influence on their mechanical properties [90, 97]. The polyaryletherketones (PAEK) are suitable, stable alternative framework materials that are used in the CAD/CAM-manufacturing of denture frameworks for complex removable dentures for patients with allergies against metals [33]. PAEKs belong to the family of high-performance thermoplastics, which were introduced to the dental market in 2006 [90, 94]; prior to that, they were used during spinal surgery for instance. PAEK materials have improved mechanical properties [73, 97], low weight [33] and a low interaction with biological materials, which contributes to their low allergenic potential [113]. However, the capacity to repair or extend PAEKs is low and they scratch faster than PMMA [41]. Furthermore, to date, there is hardly any clinical data on the long-term performance of these materials in an oral cavity. Regardless of the material, CAD/CAM-manufacturing allows the easy reproducibility of dentures in case of loss or damage thanks to the stored CAD/CAM data. Also, denture modifications such as relining can be made digitally and the dentures can then be manufactured again [90].

Since the supporting alveolar bone for a denture changes in the course of the wearing period, relining to improve mastication and reduce pressure points may be indicated. For this purpose, a distinction is made between rigid relining materials such as cold curing PMMA and soft relining materials based on silicone or acrylate [52, 85]. The latter group of materials is mainly used for removable denture relining in cases of unfavorable morphology of the alveolar process; examples include strongly undermined alveolar ridges, flabby ridges or strongly atrophied alveolar ridges with an exposed inferior alveolar nerve [16]. Moreover, these materials are indicated in situations that require minimal load on the

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**Table 1** Overview of different processing forms of polymer materials with examples
denture supporting tissues such as after surgical interventions (e.g. extractions or implant insertion).

1.3 Biofilm formation
The oral cavity provides habitat for a variety of microorganisms, with bacteria and fungi being the main colonizers of teeth, mucous membranes and dentures (see Fig. 3). Over 700 different types of bacteria have been identified as components of the oral microbiome [50]. Before bacteria or fungi attach themselves to teeth or dental restorations and form biofilms, a so-called acquired pellicle develops on all natural surfaces of the oral cavity and on the surface of dental restorations within seconds to minutes after cleaning [37, 46, 104]. The pellicle consists mainly of proteins (including enzymes), carbohydrates and lipids derived from saliva, gingival sulcus fluid or bacteria [38]. Their formation is initially based on electrostatic interactions. The phosphate ions contained in saliva contribute to the negative charge of teeth and dentures; the positively charged calcium ions, which are also present in saliva, are therefore attracted via electrostatic forces and embed proteins (e.g. phosphoproteins, statorin, histatin) in between the ion layers. Additionally, Van der Waals forces and protein-specific charged functional groups increase the adhesion of the initial pellicle to the surface of teeth and dentures [105, 106]. Furthermore, the subsequent coupling of protein aggregates from saliva via protein-protein interactions with the already immobilized proteins of the initial pellicle follows.

Pellicles display different ultrastructures and thicknesses depending on their location, with these being mostly determined by the salivary biopolymers present at the respective location and the existing shear forces, but less by material-related parameters [36]. However, the material itself influences the composition of the pellicles. For example, fewer statherines and histatines, which are responsible for defense, are found on denture materials [22]. At the same time, the pellicle can hide the properties of the underlying substrate [28, 35]. Other than serving to lubricate and protect tooth surfaces, pellicles play an equally important role for microbial attachment to teeth and removable dentures. Components of the pellicle serve as receptors for the attachment of microorganisms. Initially, mainly Gram-positive streptococci (e.g. Streptococcus oralis, Streptococcus sanguinis, Streptococcus mitis) and rods (e.g. Actinomyces naeslundii or oris) colonize the pellicle, thus making them among the early colonizers. As the bacterial biofilm matures, further microorganisms are integrated into the biofilm over a period of days. Gram-negative cocci (e.g. Veillonella spp.) attach themselves to the early colonizers at first. Then, they are followed by Gram-negative, filamentous species such as the bridge germ Fusobacterium nucleatum and late colonizers (e.g. Capnocytophaga sputigena, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Treponema denticola, Tannerella forsythia, Prevotella intermedia), some of which are leading germs of oral infections [54, 61, 62]. Fungi such as Candida albicans can also interact with bacteria such as Streptococcus gordonii, S. oralis, S. sanguinis [57, 81], A. oris [31] and F. nucleatum [30] and take part in the complex oral biofilm community [112]. Yet, the presence of specific pathogens alone is not sufficient for the development of diseases in the oral cavity. Instead, the dynamic interactions between the microorganisms and the host organism, particularly the host’s immune defense, play a decisive role in the development of biofilm-associated diseases. Diseases that can be caused by oral microorganisms include both local manifestations as well as systemic diseases.

1.4 Local diseases caused by biofilms in denture wearers
The fungus C. albicans is of particular importance in this context, as it plays an essential role in the development of denture-related stomatitis [10]. Wearers of complete dentures are more likely to develop denture-related stomatitis than wearers of partial dentures [1], which is most likely resulting from the larger interface. Denture-related stomatitis has a prevalence of up to 75 % [27]; it manifests itself as local redness of the mucosa that is covered by the denture and is often accompanied by burning, discomfort, impaired taste or pain. The development of denture-related stomatitis is dependent on
several favoring factors. Inadequate oral and denture hygiene, wearing of the denture all day with the associated reduction in the pH value of the oral mucosa to below 6.5, as well as a weakened immune system can promote the manifestation of C. albicans [27, 63]. In this way, the virulence of C. albicans appears to grow with increasing biofilm maturation, as the fungus undergoes a morphological transformation from predominantly blastospores to hyphae [98]. Studies have revealed that the material surface can also trigger the transformation of blastospores into hyphae [16, 20, 87]. The latter microorganisms are able to invade the affected mucous membrane areas with the help of enzymes and penetrate deeper into mucous membrane layers [10, 59, 98]. Aspartate proteinases, in particular, appear to accelerate the degradation of host proteins and thus promote the invasion of C. albicans [42]. Studies have proven that the activity of proteinases correlates with the severity of denture-related stomatitis [89]. Moreover, C. albicans which were organized in biofilms showed higher aspartate proteinase secretion levels than planktonic C. albicans [68]. This fungus, like other microorganisms, can also degrade material surfaces, which leads to material roughening and the further irritation of the mucosa [87].

1.5 Systemic diseases triggered by biofilms in denture wearers

In recent years, numerous studies have shown that microorganisms in the oral cavity can substantially influence and promote the development of systemic diseases. Oral infections such as periodontitis lead to cell aging (senescence): in comparison to healthy patients, the telomerase activity of affected patients is increased and cannot be reduced, or only slightly reduced, by protective measures such as exercise [67]. Other studies have identified oropharyngeal bacteria in atherosclerotic plaques [5, 21, 69], which suggests that bacteria can enter the bloodstream via the periodontal support apparatus, and thus, promote the development of cardiovascular diseases. With regard to the importance of biofilms on removable dentures, respiratory pathogens have been detected in biofilms on dentures [82, 103], which confirms an association between the occurrence of pneumonia and the wearing of removable dentures [23, 43]. The presence of respiratory pathogens in biofilms on teeth and dentures seems to be related to the pathogenesis of nosocomial pneumonia, but also to the initiation or progression of chronic obstructive pulmonary disease [91]. Pneumonia is one of the most common diseases in the elderly population and, with a mortality rate of 25%, is one of the most frequent causes of death [76, 95]. In particular, swallowing disorders (dysphagia), wearing dentures at night, inadequate denture hygiene and a weakened immune system favor the development of aspiration pneumonia [71, 91]. Besides aspiration pneumonia, gastrointestinal infections belong to possible disseminated infections caused by the accumulation of oropharyngeal bacteria on denture surfaces [77]. Various studies have shown that improved oral hygiene, with the accompanying lower germ load, has a positive effect on morbidity and mortality from pneumonia: In this manner, 10% of pneumonia-related deaths in nursing homes could be prevented by improved oral hygiene [95]. Optimized oral hygiene also seems to be more effective in reducing pneumonia-related mortality rates than drug therapy. Besides this, patients with improved oral and denture care experienced a shorter fever duration than patients who did not intensify oral and denture hygiene [109].

2. Modern materials and strategies for modulating biofilm formation and removal from removable dentures

In the development of new dental materials, the optimization of mechanical properties such as flexural strength, resistance to fracture or hardness and the improvement of the esthetic appearance are often the major focus. However, the above-mentioned considerations concerning the prevalence and importance of biofilms on removable dentures show that strategies, which minimize the adhesion of biofilms to removable denture materials, or allow easy removal of these biofilms from the surface of the dentures, could contribute significantly to maintaining the oral and systemic health of denture wearers. For this reason, in addition to optimizing the mechanical and esthetic properties of denture materials, biological considerations should also be taken into account when these materials are further developed.

2.1 Modification of biofilm formation on removable dentures by means of material properties

For the adhesion of biofilms on polymeric materials, it seems that their chemical composition, in particular, as well as their surface roughness, energy and topography are relevant properties. In general, their influence decreases with increasing biofilm thickness [35]; this substantiates the idea that a potentially preventive influence of the material must be maintained by regular mechanical removal of the adhering biofilm. This further implies that innovative material-associated strategies for controlling biofilms on polymeric materials for the production of dentures must have sufficient resistance to withstand the necessary repeated mechanical cleaning.

A high surface roughness generally causes an increased accumulation of microorganisms due to the increased surface area available for adhesion and the furnishing of niches protecting against shear forces, which can in turn be decreased by polishing. Although macrouflled resin composites of earlier generations, especially, were associated with high surface roughness, and thus high plaque accumulation, modern hybrid resin composites show much better behavior in this regard [44]. However, different degrees of biofilm adhesion were observed for different CAD/CAM materials despite comparable surface roughness. The group of polymers showed the lowest biofilm adhesion: Polymer materials such as denture base materials have a larger proportion of organic components, which presum-
ably cause less bioadhesion than inorganic components [4]. To date, there have been very few studies regarding the accumulation of biofilms on modern materials for the CAD/CAM-fabrication of removable dentures. Lower surface roughness values and lower adhesion of C. albicans have been demonstrated for PMMA processed by CAD/CAM than for PMMA produced by conventional methods [72]. Hence, it can be assumed that, in addition to improved mechanical properties, biofilm adhesion is also lower for removable dentures fabricated using CAD/CAM technology as compared to conventional fabrication of polymer materials [83, 97].

The chemical composition of polymeric materials also appears to play an important role in the adhesion of microorganisms. The addition of antibacterial substances to dental materials can be one means of delaying or minimizing biofilm adhesion and growth. Possible antibacterial additives include silver ions [15, 108], zinc oxide nanoparticles [101] and chlorhexidine [60]. The best known antibacterial dental material is amalgam. However, the example of amalgam shows that the development of effective antibacterial materials is always a balancing act between antibacterial [9, 40] and cytotoxic effects [64]. Furthermore, the release of antibacterial substances has the disadvantage of having a temporary effect. Substances which are added, or more specifically, their release can have a negative influence on the mechanical properties [2, 51, 110]. It has been shown that with increasing polymerization time of resin composites, and/or light based on the manufacturer's instructions is strongly recommended. In recent years, the processing and machining of dental materials such as PAEK or PMMA by means of CAD/CAM-processing has become established. Unfortunately, there are only a few studies investigating biofilm formation on PAEK materials [96]. Some studies have presented a lower bioadhesion on PAEK materials than, for example, on conventionally processed PMMA [35, 70]. To date, however, it has not been conclusively clarified which mechanism is responsible for this finding. One assumption is the more homogeneous composition and high curing degree of CAD/CAM vs. conservatively processed materials.

Studies on the effect of the surface topography of dental resin composites on the adhesion of microorganisms show that microstructured surfaces are more hydrophobic due to higher water contact angles, thus resulting in increased air inclusions, which in turn reduces the total available contact area between materials and microorganisms [25]. In addition, the topographic barriers lead to a reduction in Quorum Sensing between the microorganisms [25]. For direct dental restorations, this effect can be exploited by using microstructured matrices for filling placement. With the aim of optimizing polymeric materials for indirect dental restorations, special polishing regimes are conceivable that leave a specially structured surface. Studies have shown that different polishing regimes, which produce diverse surface patterns, tend to have different degrees of bioadhesion, even if they have a comparable final roughness [34, 44, 86]. With regard to denture bases that are not polishable, the fabrication of removable dentures using CAD/CAM-processing could be interesting, since these materials appear to exhibit positive properties with regard to biofilm adhesion [72]. While the surface topography of polymers can be modified by polishing and production methods, biomimetic microstructuring of metals is possible with the aid of special lasers and this has shown reduced microorganism attachment [3, 18]. Thus, the surface structuring of metal denture frameworks using laser offers a prospect for the further development of dental biomaterials.

The effects of different denture materials and their surface properties on bacterial adhesion and biofilm formation have not yet been sufficiently characterized. However, the elucidation of the underlying mechanisms could make a significant contribution to the future optimization of denture materials from a biological point of view; the aim would be to reduce the prevalence of biofilm-induced diseases in denture wearers in the long term. For both approaches, elucidation of mechanisms and development of innovative denture materials, reproducible model systems close to clinical practice can be used; the oral multi-species biofilm model can, for example, be used for in-vitro studies under static and under dynamic flow conditions that resemble clinical practice [55, 56]. This model is already being used in dental implant research [19]. Such in-vitro analyses, which are frequently performed by means of high throughput screening, should be complemented, or validated, by in-situ studies, such as by placing test specimens in splints or dentures. In-situ approaches have the advantage of allowing biofilm formation to occur under the natural conditions of the oral cavity.

2.2 Modification of the adhesion of Candida albicans on removable dentures through material properties

Since denture bases are usually not polished and the denture plastic can be penetrated by C. albicans [66], the rebasing or the new fabrication of the denture is advisable, especially for older dentures and existing denture-related stomatitis, so as to avoid reinfection after antifungal therapy of the mucous membranes [58]. It is known that C. albicans reacts less sensitively to antifungal therapy, particularly in pores of rough material surfaces [102] and leaves endotoxins in these pores, which further sustain the infection by slow release [16]. A reduced attachment of C. albicans occurs on smooth and hydrophilic surfaces [29, 75, 88, 100, 111]. Additionally, a relationship between the basic part of the surface free energy and the adhesion of C. albicans could be demonstrated [49]. Furthermore, it has been shown that the adhesion of C. albicans to polyamides is higher.
than to PMMA-based resins [24]. With regard to the different materials available, there are contradictory results concerning the adhesion and proliferation of C. albicans: While some authors demonstrated a significantly higher Candida colonization of PMMA than on silicone-based soft relining materials [80], other authors were able to demonstrate a lower colonization of PMMA with C. albicans as compared to the soft relining materials [6]. A possible explanation for these varying results could be related to the porosity of soft relining materials, which may harbor a large number of Candida cells in their pores and make them inaccessible for analysis, thus conceivably falsifying the results [80]. It could also be shown that materials with high surface energies such as urethane dimethacrylate (UDMA) and silicone displayed higher colonization with C. albicans than materials with comparatively lower surface energies [53]. The proportion of hyphae on silicone-based materials was higher than on UDMA- or PMMA-based materials [98].

In this regard, it is worth considering that most of the present investigations, especially with respect to the analysis of the adhesion of C. albicans to different denture base materials, have been carried out under experimental conditions; this means that the settings are often not very comparable and this is further complicated by the fact that clinical investigations barely exist. In spite of this, based on the available data, it can be concluded that for the production of denture bases, hydrophilic materials should be used as far as possible, as well as materials that have the lowest possible initial roughness after production; in this manner, porosities, and thus niches for biofilm formation, can be minimized in order to reduce biofilm-associated diseases.

3. Microorganisms change materials

Every material that is introduced into the oral cavity is subject to an ageing process as a result of use. The surfaces of removable dentures are no exception and they show signs of ageing and fatigue due to the daily mechanical, thermal and chemical stress during use and cleaning [90]. In the long term, this can lead to surface roughness, discoloration and odor. In addition, the moisture in the oral cavity and the moist extraoral storage environment cause the material to absorb water, which varies in extent depending on the material, and can lead to a reduction in the strength of the material [99]. It appears that thermoplastics absorb less water than cured resin materials [45]. Drying of the denture in turn can lead to distortion and a reduced accuracy of fit, although, shorter drying phases can reduce the formation of bacteria on the surface of the material [90]. In addition, microorganisms play a decisive role in the modification of polymer denture materials [8, 26, 39, 84]. Even pellicle intercalation between the matrix and filler material can cause fillers to dissolve out of the resin composite, and thus, favor the polymer’s deterioration. Some of the enzymes that are secreted by microorganisms [13], in addition to acids, can degrade material surfaces [12, 65]. This can increase the surface roughness of the materials [74], which on the one hand, promotes bioadhesion, while also simultaneously irritating the mucosa in contact. This phenomenon seems to affect the polymer materials of older generations especially [74]. Therefore, the use of newer generation polymer materials as well as regular professional cleaning and polishing of polymer-based restorations seem to be recommendable. However, no clinical or experimental data is available to date regarding the long-term durability of modern materials that are used for the fabrication of removable dentures such as PAEKs or CAD/CAM-processed PMMA [96].

4. Future prospects

Removable dentures will play an important role in dental prosthetics in the foreseeable future. Due to the current demographic trends, an increasing number of older patients are being treated with dentures. Since regular and adequate removal of biofilms from the surface of removable dentures cannot be ensured in all cases, it would be desirable to develop materials and strategies that make the biofilm accumulation on, and the removal from, denture surfaces manageable and predictable. Currently, the available data from clinical studies regarding the interaction between polymeric materials of removable dentures and biofilms is rather sparse. The first reported results for modern polymeric materials with optimized material properties have been promising. Further strategies that promise the easy removal of adherent biofilms from the surface of denture base materials have so far only been described in very limited laboratory studies, mostly with a different background. At the moment, research regarding clinical applications is still pending.

Conflicts of interest

The authors declare that there is no conflict of interest within the meaning of the guidelines of the International Committee of Medical Journal Editors.

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