J Incl Phenom Macrocycl Chem (2007) 57:297–302
DOI 10.1007/s10847-006-9176-0

ORIGINAL ARTICLE

Guided tissue regeneration membranes with controlled delivery properties of chlorhexidine by their functionalization with cyclodextrins

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Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 16 January 2007

Abstract In parodontology, guided tissue regeneration (GTR) is a new technique to cure periodontal lesions. Where the association of the GTR with an antimicrobial agent does not yield optimal results, we used the properties of cyclodextrins (CDs) to improve the membrane used in RTG to control the release and to increase the quantity of antimicrobial agent stocked on the membrane. We successed in fixing 14%-wt of cyclodextrin polymer on polyvinylidene difluoride (PVDF) membranes thank to citric acid (CTR) as crosslinking agent. We studied the complexation of chlorhexidine diacetate (CHX), the antiseptic agent used in this study, with CDs in UV-spectrophotometry and ROESY NMR. We observed complexation of CHX by β, γ, hydroxypropylated (HP) βCD. We studied the biological properties of the cyclodextrin polymer onto (PVDF) membranes and observed that the CDs-polymer is not harmful for the cells. Moreover it stimulates their growth with native CD. A kinetic of release of the CHX was performed. Raw membranes released all CHX stocked in few hours, whereas grafted membranes released more than tenfold this quantity during 60–80 days.

Keywords Biomaterials · Cyclodextrins · Drug delivery system · Periodontal · Polycarboxylic acids · Tissue regeneration

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Introduction

The periodontitis is a disease caused by the dental plaque. A biofilm of bacteria migrate to the epithelio-connective structure and destroy the alveolar bone and the periodontal ligament and forms what is called a periodontal pocket. Progressively, the periodontal ligament is replaced by the epithelium tissue and the attachement of the tooth to gengiva is affected. The consequence is the receding of the gums. Classic methods to cure this disease is the cleaning of the area to eliminate the infection. Nevertheless, the epithelio-connective attachment is not restored : a junctional epithelium recovers the root [1–4].

A new method called the guided tissue regeneration (GTR) is proposed to improve the results.

A membrane is placed between the epithelium and the alveolar bone to promote the regeneration of the later. Studies shows good results of this technique but does not ameliorate the attachment because membranes are colonized by bacteria decreasing the efficiency of the treatment. Besides, the association of an antimicrobial agent does not increase significantly the regeneration. The reason is that the quantity of

antisteptic agent stored onto the membrane is too low in the area and the antimicrobial activity does not last [5–8].

To release drug in an efficient quantity for a long time, we propose to use the complexation properties of the cyclodextrins (CDs). As we previously reported, it is possible to modify PVDF membranes by CDs with the use of citric acid as crosslinking agent [9–12].

We studied the properties of (PVDF) membranes functionalized by CD. We also observed the biological properties of the functionalized membranes and the kinetic of release of the chlorhexidine, a largely used antimicrobial agent in the periodontal domain [13, 14].

Materials and methods

Materials

Porous Polyvinylidene fluoride (PVDF) membrane (pore size : 5 μm) was supplied by Millipore®. βCD was a gift from Roquette (Lestrem, France), γCD, Hydroxypropylated βCD (HPβCD) and Hydroxypropylated γCD (HPγCD) were gifts from Wacker Fine Chemicals GmbH (Burghausen, Germany). Citric acid (CTR), sodium dihydrogen hypophosphite and chlorhexidine diacetate (CHX) were Aldrich chemicals (Milwaukee, WI, USA). The textile finishing equipment consisted of a padder and a thermofixation oven (Roaches, Leek, UK).

Methods

Functionalization of PVDF membranes by cyclodextrin

Fabrics were impregnated by the reactants (100 g l^{-1} CTR/30 g l^{-1} sodium dihydrogen hypophosphite/ 100 g l^{-1} CD) dissolved in water, roll-squeezed, dried and thermofixed (at variable temperature and time) and washed by a soxhlet extractor for 210 min with water. Raw and treated samples were dried 60 min at 104°C before being weighted. The grafting rate of the modified membranes was calculated by the following equation: %-wt = $(m_f - m_i)/m_i \times 100$. The precision on the weight gain measurements was (±1.5 %-wt.

Characterization of the complex inclusion of chlorhexidine diacetate into cyclodextrin: NMR spectroscopy

CD was solved in D_2O and CHX was solved in DMSO. These solutions were then mixed to be studied by ROESY NMR in a BRUKER advance 400 MHz.

Biocompatibility of the PVDF- membrane

Membranes were cut in disk then sterilized thanks to UV irradiation for 2 h.

The cells used to study biocompatibility were L132 cells (human embryonic pulmonary tissue).

The disks were placed in polystyrene wells and with 10^5 L132 cells in 1 ml of culture medium. Positive control are obtained with the bottom of the well filled with culture medium and cell. Negative control are obtained by filling wells with culture medium containing cells and by adding nickel powder. Sample are stocked at 37°C in an atmosphere with 5% of CO_2.

For the proliferation test, the cells were counted after 3 days and 6 days of culture with a coulter ZI cell counter. For the vitality test, after 3 and 6 days of incubation, 500 μl of a dye, Blue Alamar was placed in wells. After three more hours of incubation, the spectroscopic absorption was evaluated at 560 nm representing the activity of cells.

In vitro release of chlorhexidine diacetate

Raw and grafted membranes were dipped in solutions of chlorhexidine diacetate for 4 h. The membranes are rinsed with distilled water and placed in batches filled with 50 ml of a buffer at pH = 7,4. The batch are shaken at 37°C.

The release of the CHX is studied by UV-spectrophotometry at 255 nm.

Results and discussion

Functionalization of PVDF membranes by cyclodextrin

The Fig. 1 and Fig. 2 show that the CDs grafting are time and temperature dependent. In Fig. 1 one can observe grafting above a threshold temperature of 130°C for HPCDs. Native CDs are less reactive than hydroxypropylated CDs as their threshold grafting temperature was 140°C. HPCDs reach their maxima at lower temperature than native CDs. We obtained an average plateau rate of grafted CDs around 14%-wt.

The Fig. 2 shows that the higher was the temperature, the lower was the time to get the maximum grafting rate. For temperature under 130°C, no gain of weight is observed

The SEM micrographs Figs. 3 and 4 show the coating of polymer around the PVDF fiber. The porosity of the membrane decreased after functionalization.

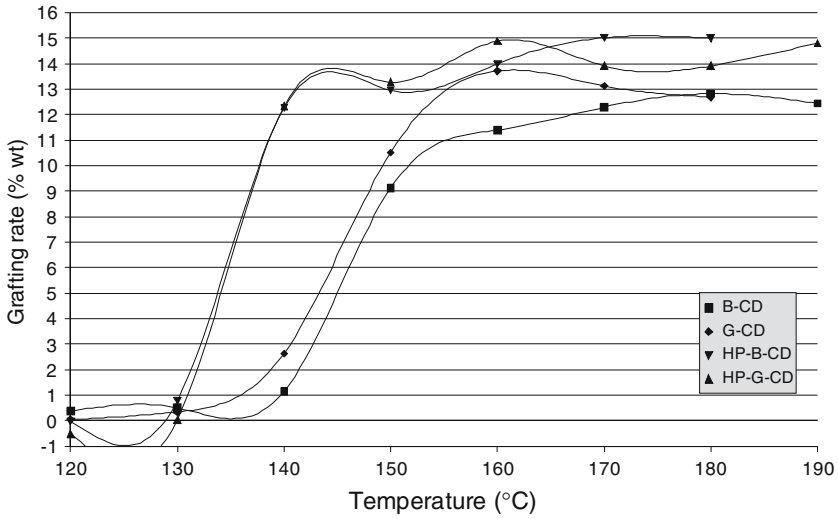

Fig. 1 Grafting rate of CD according to the temperature. Curing time = 10 min

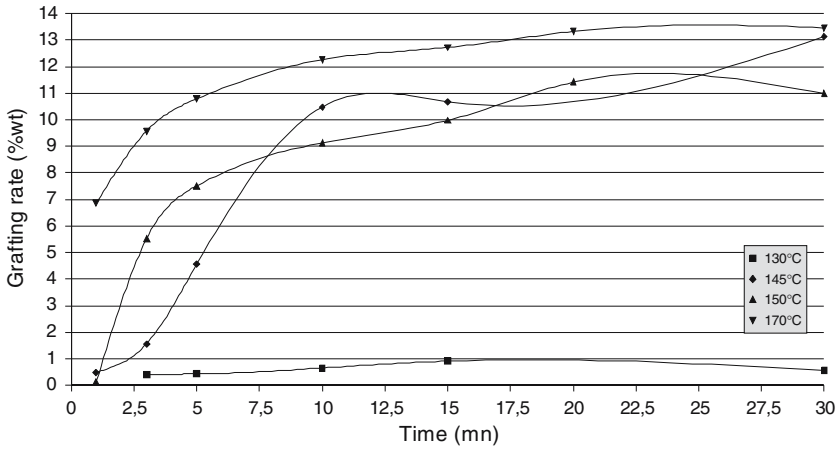

Fig. 2 Grafting rate of βCD versus time of curing

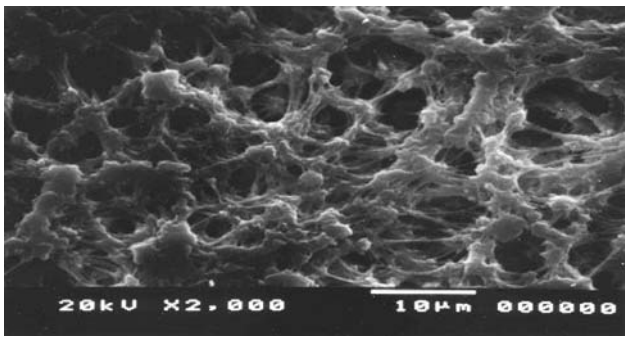

Fig. 3 SEM micrographs of ungrafted (×2000)

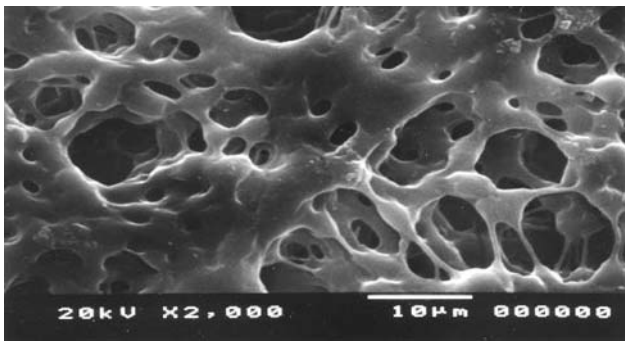

Fig. 4 SEM micrographs of grafted membranes (×2000)

Characterization of the complex inclusion of chlorhexidine diacetate into cyclodextrin : NMR spectroscopy

The ROESY NMR of the mixture of βCD/CHX (Fig. 5) shows correlations between the inner protons H3 and H5 of the CD and the aromatic protons of CHX. The existence of a complexation by inclusion between the β-CD and the aromatic group of CHX. Similar results can be observed with γCD and HPβCD.

However, no correlation was observed with the HPγCD. So, we cannot observe any inclusion of the CHX into the HPγCD cavity.

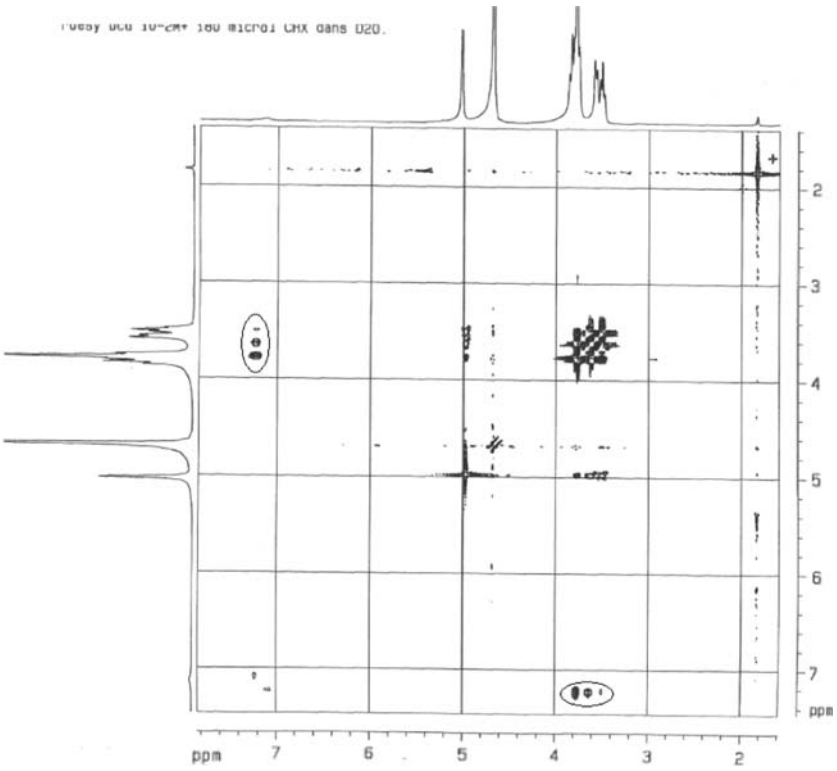

Fig. 5 ROESY NMR spectrum of mix βCD/CHX

Biocompatibility of the PVDF-membrane

The proliferation tests (Fig. 6) show that after 3 days, proliferation of cells on raw membranes is at 50% of the control. Cells on grafted membranes present an increase of the proliferation and especially for the HPCDs. After 6 days, proliferation is improved and reaches level equal to control for raw membranes and membranes with native CDs. HPCDs showed a little decrease of the number of cells.

The vitality shows (Fig. 7) good results for all membranes. At 3 days, cells on the membranes show as intensive vitality as on the control. At 6 days, a decrease of the vitality is observed for cells on raw and HPCDs grafted membranes. On the contrary, cells on membranes grafted with native CDs give vitality near to control.

Proliferation and vitality tests shows that the best result are obtained on membranes grafted with native CDs.

The kinetic of adhesion (Fig. 8) of the cells showed that results are similar to the control. These tests, in addition to proliferation and vitality tests, prove that PVDF membranes modified with CDs are biocompatible and can be used in the GTR technique.

In vitro release of chlorhexidine diacetate

As observed in Fig. 9, the raw membranes released only 1 mg g^{-1} in a few hours, whereas membranes

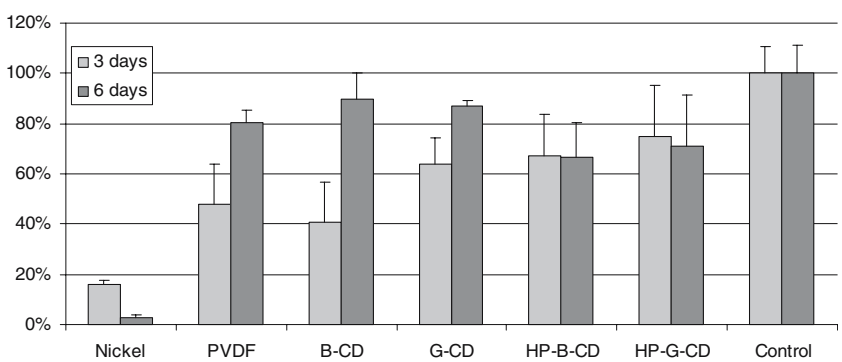

Fig. 6 Proliferation tests of L132 cells on PVDF membranes

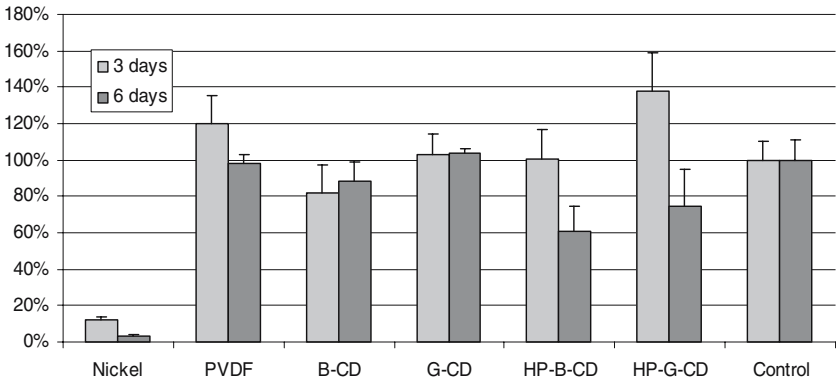

Fig. 7 Vitality tests of L132 cells on PVDF membranes

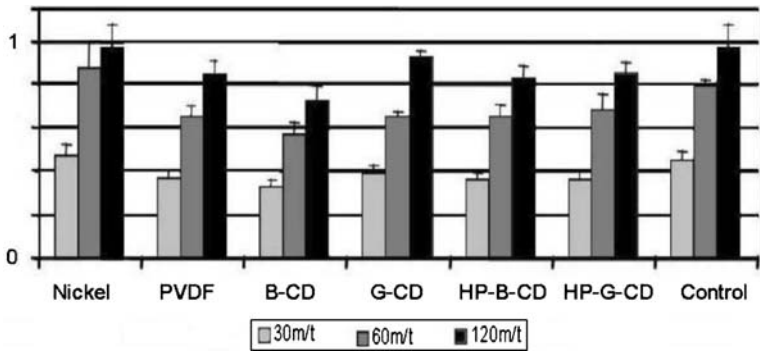

Fig. 8 Adhesion tests of L132 cells on PVDF membranes

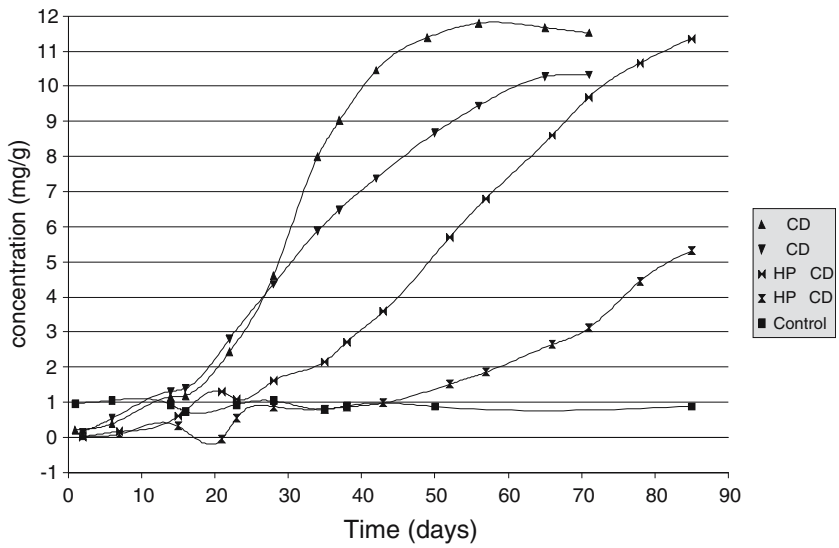

Fig. 9 In vitro release of CHX (displayed in mg of CHX per gram of membrane) from virgin and CDs grafted membranes in batch containing distilled water

grafted with βCD, γCD and HPβCD released upto 12 mg g^{-1} of drug within the experiment period. Membrane grafted with βCD released the antiseptic agent during 55 days. It lasts 65 days with membranes grafted with γCD and 80 days with membranes grafted with HPβCD. However, HpγCD grafted membranes released lower quantity of CHX compared to other CDs. This is in correlation with NMR results.

Conclusion

We showed that we can effectively fixe CD onto PVDF membranes and control the grafting rate by the adjustment of time and temperature of grafting. NMR spectroscopy proved the inclusion of CHX into βCD, γCD and HPβCD, but not with HPγCD. The surface of grafted membranes is favorable to the proliferation, the vitality and the adhesion of the L132 cells.

Biological properties of the PVDF membranes modified with CDs are compatible with the GTR application in in vivo conditions. The In vitro study of release showed that we managed to release a higher quantity of antibacterial agent for a long time thanks to CDs, except with HPγCD, that does not complex CHX as supposed with ROESY NMR spectroscopy.

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