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Ultra-Thin Layers of Phosphorylated Cellulose Derivatives on Metal Surfaces

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Summary: Cellulose as natural and non-toxic material is very interesting for biological applications. Its poor solubility in organic solvents can be improved by introduction of long alkyl chains. Phosphate-substituted cellulose derivatives form ultra-thin layers on several metal surfaces [1, 2]. The layers were applied on the surfaces via dip coating from dilute solutions and characterised by contact angle measurements. Initial corrosion tests were performed. These polymers were applied as adhesion promoters for steel-, titanium- and hydroxy apatite surfaces. Medical implants were pre-treated by UV irradiation, coated with the cellulose derivatives and activated by hot water treatment. The adhesion of the implants surpassed that of conventionally used PMMA systems.

Introduction

The present work deals with the application of biocompatible cellulose derivatives on implant materials in medicine.

Cellulose shows some very interesting properties: On one hand it is non-toxic, has a good thermal stability and due to its hydroxyl groups a high polyfunctionality. On the other hand cellulose possesses poor solubility in organic solvents and low thermo plasticity. And, natural celluloses do not form stable and ordered layers on metal surfaces.

The poor solubility of cellulose in technical solvents can be improved by introduction of alkyl chains. These polymers consist then of rigid main chains and liquid-crystalline side chains (see figure 1)

Wegner et al. investigated Langmuir-Blodgett layers formed from so-called ”hairy-rod” polymers on the basis of cellulose derivatives, which possess hydrophobic side chains with a length of 4 –5 methylene groups.

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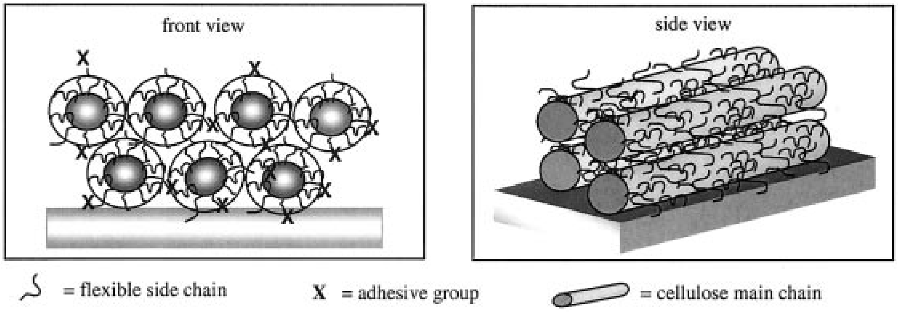


Figure 1: Scheme of "hairy rods" in side and front view

These "hairy-rod" cellulose polymers are able to form mono- and multilayers on hydrophobic surfaces [3-6]. It was possible to get regenerated cellulose with high biocompatibility by hydrolysis of cellulose silylesters [3].

Due to the easier handling required for the self-assembling technique [7] it was our intention to synthesise functionalised cellulose derivatives that are soluble in water or ethanol [8]. The functionality can be reached by introduction of functional groups into the terminal positions of the side chains. A synthetic route was developed to phosphorylate water-soluble hydroxypropyl cellulose [9-11].

The introduction of phosphate groups into the cellulose was the premiss for developing a system that was able to solve several adhesion problems in medicine. Main problems are that implants often loose the support to the bone and that tissue inflammation occurs. This can be solved by using modified layers between implant and bone to improve adhesion and minimize inflammation by incorporation of antibiosis. Implants of steel or titanium have been bond via the modified cellulose derivative to the bone (hydroxy apatite).

Thin films from phosphorylate hydroxypropyl cellulose were formed using the self-assembling technique on aluminium and titanium. We could observe a good adhesion on these substrates caused by the effect of the phosphoric acid groups [1, 2].

The characterisation of the films was carried out with contact angle measurements. Adhesion promoters for implant materials in medicine should not cause any corrosion. Therefore, initial investigations on the corrosion inhibition capability on aluminium surfaces were carried out using the constant humidity climate test (DIN 50017) for pre-treated metal plates. A second test was carried out in which the metal plates (Al, steel, Ti) were pre-treated with the cellulose derivatives and then coated with a clearcoat that was then damaged to assess the infiltration. Further investigations were made to stabilise the films of phosphorylated cellulose derivatives with a matrix of cross-linkable cellulose derivatives. For analytical investigations the composite was cross-linked by UV-radiation. This procedure is not usable for an application in the human body. Therefore, a new method was developed for cross-linking the composite [13].

Experimental part

All reagents and solvents were distilled and dried before using. Hydroxypropyl cellulose (Aldrich, MS = 4,8 (NMR), M_w ~ 100.000 g/mol,) was used without further purification. The synthesis' of hydroxypropyl-2-phosphatepropyl cellulose **1** [12] and hydroxypropyl-2-cinnamoylpropyl cellulose **2** [12] were described elsewhere [1].

Al substrates (AlMg1) of technical grade were provided by Chemetall GmbH, Frankfurt. The pre-treatment of the surface was published recently [12]. Ti (TiAL6V4) and steel were used after ultrasonically cleaning in acetone. Si substrates were ultrasonically cleaned in acetone for 15 minutes, immersed for one hour in a solution of H_2O/H_2O_2 (30 %)/NH_3 (25 %), ratio 5:1:1 at 80 °C, rinsed with water and dried under a stream of nitrogen.

Contact angle measurements

A K12 processor tensiometer from Krüss GmbH was used to measure the contact angles of the coated Al substrates with known geometry by the Wilhelmy method. Advancing and receding contact angles were calculated.

Constant humidity climate test [14]

This test was performed according to DIN 50017. The tests serve to clarify the corrosion protection of pre-treated aluminium plates under the influence of constant humidity at 40 °C for 96 h.

Industrial testing with clear coat top layer on aluminium substrates

The aluminium substrates were coated first with the thin cellulose derivative films and then with a commercial acrylate clearcoat (Weber + Wirth dried for 24 h in air). The coatings were mechanically damaged, activated with HCl-vapour for 1h and then exposed to the influence of constant humidity at 40 °C for 1000h. The results are described in mm infiltration.

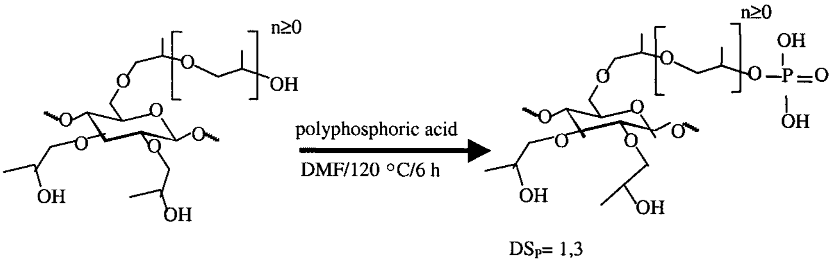
Coating

The cleaned substrates were dip-coated in solutions at various concentrations (0,1-3 %) and for different dipping times (1-24 h), rinsed with water and dried in vacuum or a stream of nitrogen.

Results and discussion

One of our aims was to use the self-assembly process for the coating of the several substrates with cellulose derivatives. Therefore, water and alcohol-soluble cellulose derivatives have been developed [1].

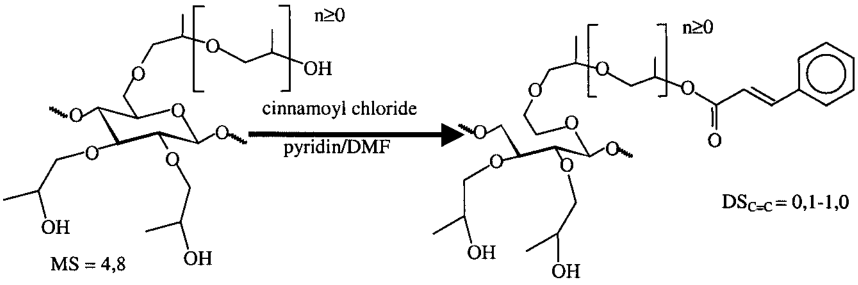
Hydroxypropyl-2-phosphatepropyl-cellulose (**1**) was synthesised via phosphorylation of hydroxy propyl cellulose with polyphosphoric acid. The degree of substitution was 1,3 (see scheme 1).



Hydroxypropyl-2-phosphatepropyl-cellulose (**1**)

Scheme 1: Synthesis of water-soluble hydroxypropyl-2-phosphatepropyl-cellulose (**1**)

The second component was a soluble cross-linkable cellulose derivative. Hydroxypropyl-2-cinnamoylpropyl cellulose (2) was prepared by introduction of cinnamoyl group into hydroxy propyl cellulose (see scheme 2).



Hydroxypropyl-2-cinnamoylpropyl cellulose (2)

Scheme 2: Synthesis of soluble cross-linkable hydroxypropyl-2-cinnamoylpropyl cellulose (2)

The degree of substitution could be varied between 0,1 and 1,0.

The adhesive and corrosion resistant effects of the cellulose derivative layers strongly depend on the layer thickness. The layer thickness was measured with ellipsometry. For these measurements it was necessary to apply the ultra-thin layers on silicon substrates because of their smooth surface (see table 1).

Solution concentration	0,1%	2%	3%
Layer thickness	1• • • • • • nm	5,9• • • • • nm	Could not be determined, too inhomogeneous

Table 1: Layer thickness of **1** on Al in dependence on the solution concentration

The thin layers of hydroxypropyl-2-phosphatepropyl cellulose **1** from dilute solutions have a thickness of one nanometer. The layer thickness got at higher concentrations indicates that multilayers have been formed.

The layer of the cellulose **1** (from 0,1 % concentrated solutions) gave constant contact angles. The contact angle measurements for the layer of **1** (from more highly concentrated solutions) were not constant (see figure 2). The layer probably either dissolves or adsorbs water. The measurements for cellulose **2** could not be clearly interpreted. It possibly demonstrates the same behaviour as cellulose **1** from highly concentrated solutions.

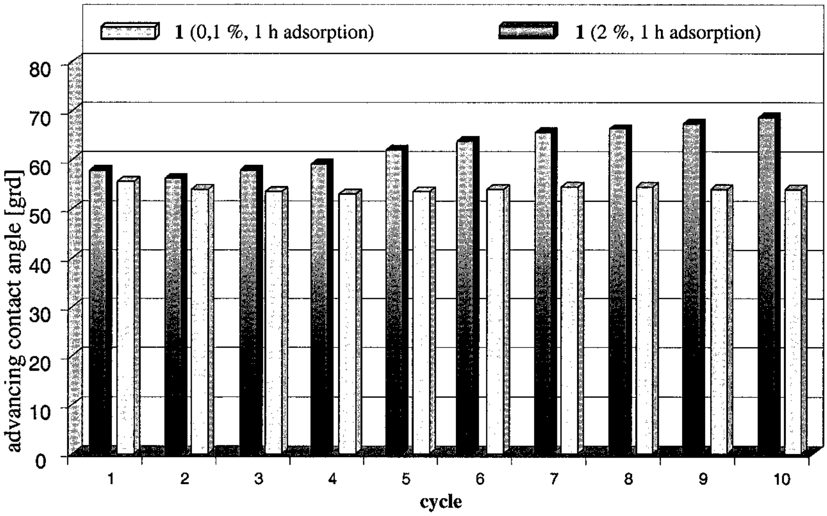
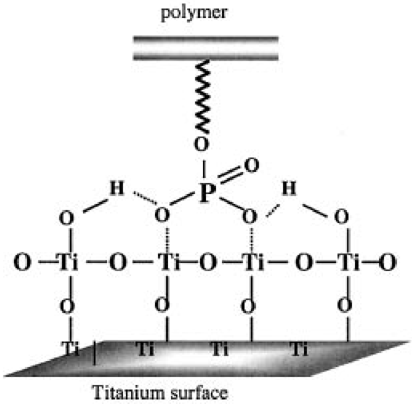


Figure 2: Stability measurements with dynamic contact angle analysis of **1** on Al



The adsorption process of phosphate groups on oxide surfaces can be considered as acid-base reactions. Figure 3 shows the model of adsorbed phosphate cellulose on the titanium surface.

Figure 3: Model for binding of phosphate cellulose with hydroxyl groups on titanium surface

Cross-linking

Cross-linking of the cinnamoyl groups occurred via a fast isomerisation to the cis-form followed by the formation of the cyclo-form via a slower reaction. A concentration of 10-20 % cross-linking groups was necessary for a stable cross-linking of the layer [2,13]. Figure 4 shows a model how the phosphorylated cellulose can be stabilised in an adhesive composite by the UV-crosslinked cellulose.

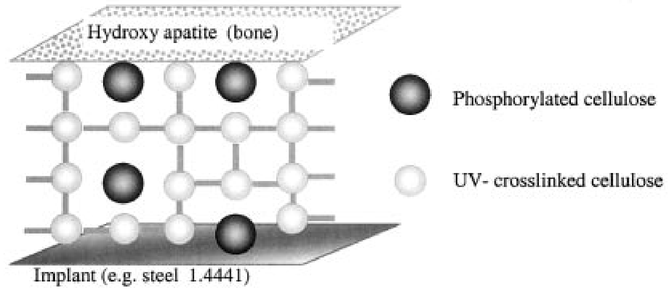


Figure 4: Model of adhesive composite

We investigated the cross-linking of the layer using UV-spectroscopy on a layer of **2** on quartz glass as shown in figure 5.

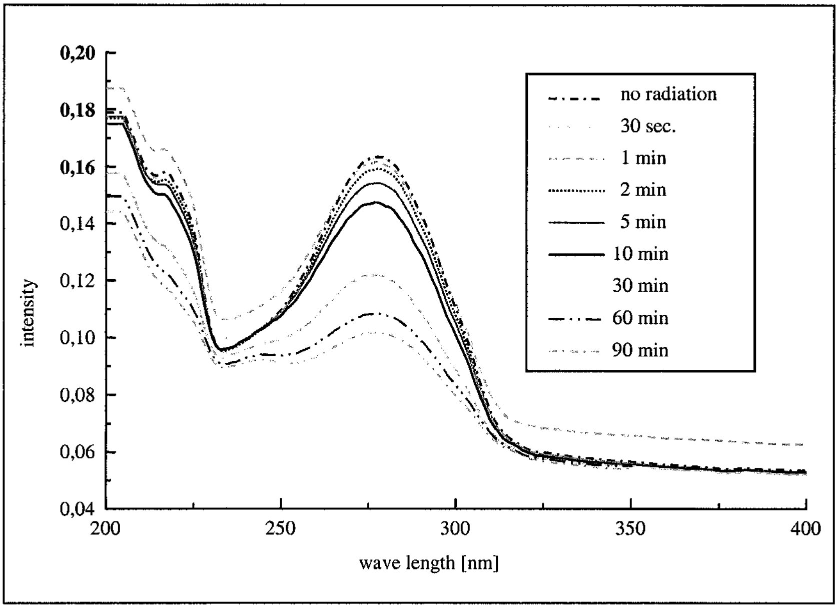


Figure 5: UV spectra of **2** on quartz glass in dependence on the radiation time

Reiser et al.[15] reported a method to calculate the amount of *trans*-, *cis*- and *cyclo*-form in the layer. The calculated amounts are shown in figure 6.

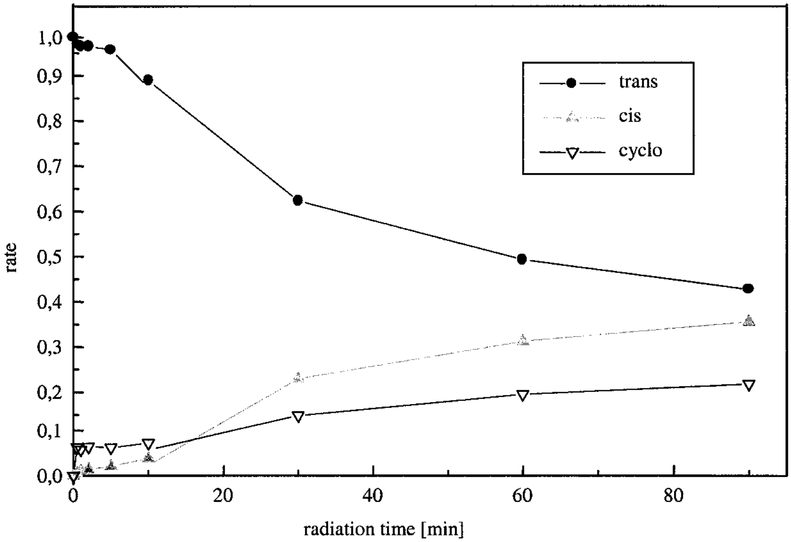


Figure 6: Rate of trans-, cis- and cyclo-form in the layer of **2** on quartz glass

After an irradiation time of 30 minutes the layer is cross-linked to 14 %, a longer radiation time only gave an increase of cross-linking to 20 %.

The stability of the layers was measured with dynamic contact angle analysis using the Wilhelmy method. Cyclic contact angle measurements give an indication of the layer stability (ten cycles for every sample). The coating is resistant to the solvent when the contact angle does not change. The results are summarised in figure 7.

The contact angles increased after the first cycle due to the change of the surface quality (dry and wet surface), after that they remained stable.

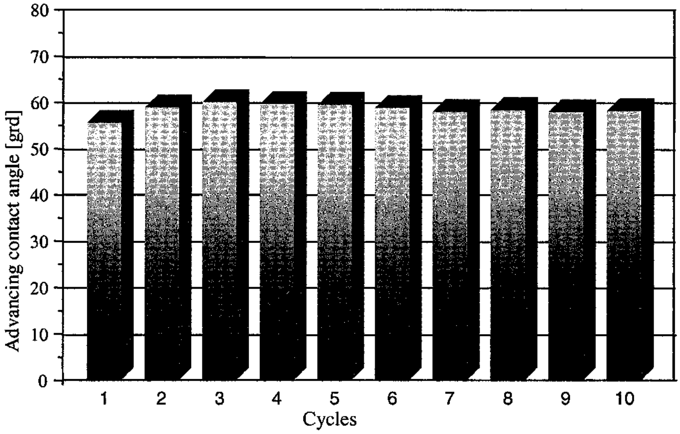


Figure 7: Cyclic contact angles of UV-cross-linked cellulose composite with high phosphate content

Corrosion tests

Fur using these cellulose derivatives in medical implant technology they have to fulfil two functions – first an adhesive and second a corrosion resistant function. Therefore, the corrosion inhibition effect of the two cellulose derivatives has been tested by constant humidity climate test and filiform test.

The constant humidity climate test showed that only the layer of **1** (0,1 % solution) provided good protection after 24 hours in comparison with a non-coated substrate. The corrosion inhibition effect is caused due to the phosphate groups. But after 96 hours there was no further corrosion inhibition. The layers of **1** (1-3% solution) and **2** show no inhibition potential (see figure 8).

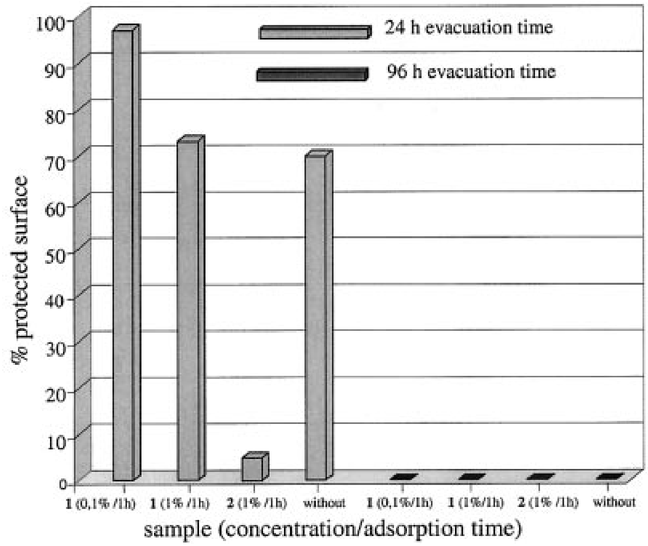
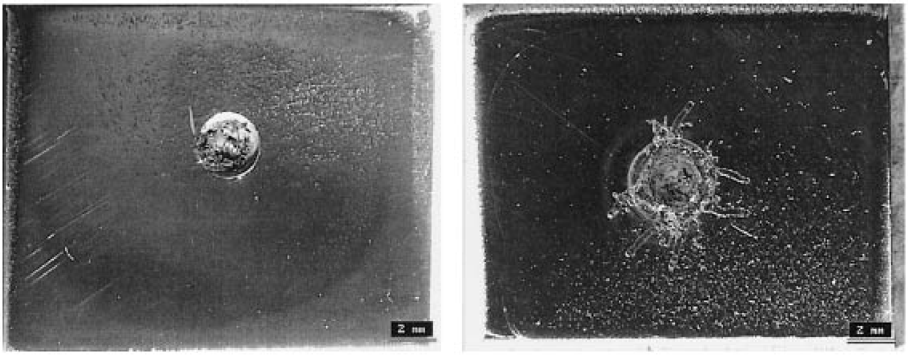


Figure 8: Constant humidity climate test of **1** and **2** on Al

The so-called filiform test is performed to investigate the tendency of filiform corrosion on scratched metal plates that were treated with the cellulose derivatives and a clear coat. The filiform corrosion tests show that the corrosion protecting effect can be observed, but it is strongly depended on the layer thickness of the cellulose derivative. The infiltration remained below 0.5 mm. The comparison of two different layer thickness' is shown in figure 9.



a) b)

Figure 9: Filiform corrosion test of coated steel substrates

In picture a) steel 37 was coated by dipping with a very thin layer of (**1**) from 0,1% solution and a following protecting coating. After 120 h exposure time there is no corrosion outside of the defect. In picture b) a thicker cellulose layer was applied under the clear coat by spin coating. In this case after 120 h exposure time distinct filaments were visible formed by infiltration of corrosion products from the defect.

New method for application under body-like conditions [2, 13]

The use of the cellulose composite as bone cement replacement requires strong fixation to the substrate under mild conditions. Therefore, a new method was developed for activation of adhesion between bone and implant. First the implant material was treated with an ethanol/water solution of the two cellulose derivatives. After drying the implant is covered with a firm cellulose film. Then this layer is cross-linked by UV irradiation. By adding water the film develops its adhesive effect. Therefore, the implant is shortly dipped into hot water (95°C). The film begins to swell without heating the metal and develops its adhesive strength. Then the composite consisting of implant, adhesive cellulose derivative and bone is fixed by pressure.

The strength of the composite was investigated with the tensile test (DIN 4624).

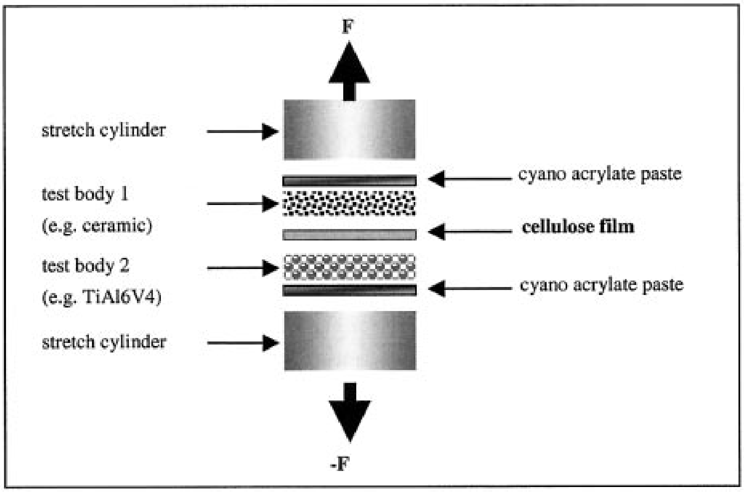


Figure 10: Set-up for measurement of adhesion effect by DIN 4624

In figure 10 the set-up for this measurement is shown. TiAl6V4, 1.4441 steel (contains 16-18% Cr, 10.14% Ni and 2-3% Mo) and hydroxy apatite were used as substrates. The substrates were coated with the cellulose derivatives, dried and then UV cross-linked. As cellulose derivatives were used different mixtures of the components: pure cinnamate cellulose, CP 91 containing 10% and CP 21 30% phosphate cellulose and pure cellulose phosphate. After activation in hot water the composite is pressed and stored 24h in air. For the tensile test a speed of 10 mm/min was chosen. The results are demonstrated in figure 11.

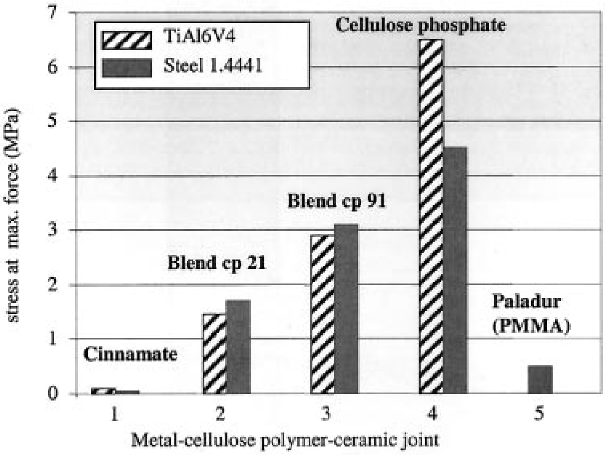


Figure 11: Comparison of different adhesive composites

From figure 11 one can conclude that any cellulose composite shows better results than the commercial product Paladur (PMMA). There are some differences between steel and titanium; the stress on the titanium substrate is 33% higher compared to steel. The adhesion between hydroxy apatite and cellulose phosphate is so strong that the break occurs in the ceramic (Fig.12).

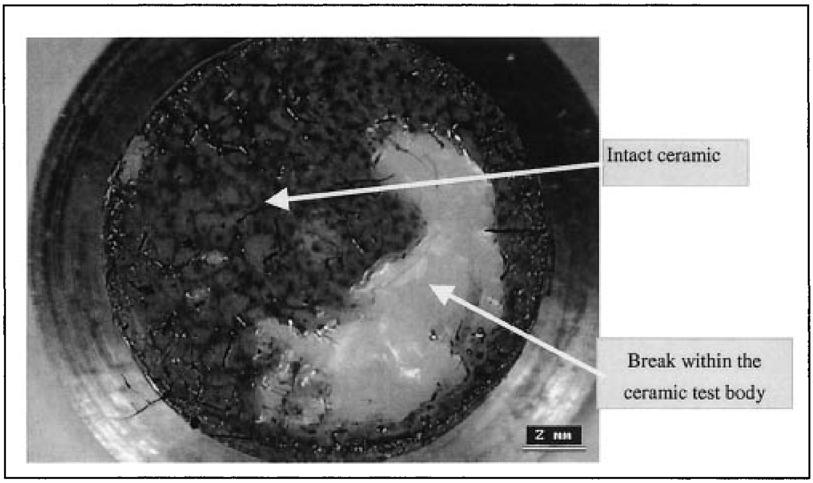


Figure 12: Front view on ceramic test body (cellulose phosphate adhesive) after tensile test (DIN 4624)

Conclusions

Two-component adhesives from different cellulose derivatives have been developed for medical implant applications. One part functions as adhesion promoter for the several substrates and the other part forms a matrix via UV cross-linking that stabilises the whole composite.

Therefore, hydroxypropyl cellulose was phosphorylated to introduce functional groups into the cellulose increasing the adhesion promotion on metallic substrates. The phosphorylated cellulose derivatives were able to build closed and smooth layers on the metal surfaces with a preferred orientation of the cellulose main chain. They showed good adhesion promotion, but no significant improvement of the corrosion inhibition without a protecting coating on aluminium. The effect of adhesion promotion strongly depended on the concentration of the adsorption solution: dilute solutions are more effective than concentrated ones.

UV-cross linked cinnamoyl cellulose was synthesized to stabilize the phosphate cellulose in a matrix.

It was possible to show that thin layers of phosphated cellulose inhibited electrochemical corrosion reactions. These layers had to be protected due to the hydrophilicity of the cellulose derivatives by a top layer. A new technique has been developed for the application of these cellulose derivatives in medicine. The activation of the adhesive was carried out by the treatment of the pre-cross-linked cellulose layer on the implant with hot water as swelling agent. We could get stable composites on hydroxy apatite ceramic, titanium alloy (TiAl6V4) and steel (1.4441).

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