Conclusion
Polymeric carrier systems in the micro- and nanometer size range proved in an in-vivo model to be a promising approach for the specific targeting to the inflamed area of gut in the case of inflammatory bowel disease. The size dependent deposition of microparticles in the inflamed tissue should be given particular consideration in the design of new carriers for the treatment of inflammatory bowel disease.

Acknowledgements
We like to thank K. Peters and J. Hoffmann (Dept. of Gastroenterology, Saarland University, Homburg, Germany) for their support during the animal experiments. The "Fonds der Chemischen Industrie" is thanked for financial support.

References

CONTROLLED PROTEINS AND Ca^{2+} RELEASE FROM BIOMIMETIC CALCIUM PHOSPHATE COATINGS ON Ti6Al4V IMPLANTS
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Introduction
A biomimetic method has been recently developed for coating metal implants with bone-like hydroxyapatite layers [1]. By controlling the physiological conditions under which the biomimetic coatings are grown from a fluid at room temperature, biologically active agents, such as bone morphogenetic proteins (BMPs) and antibiotics, can be co-precipitated. This creates the possibility to use these coatings as slow drug release systems [2,3].

The objective of this study is to (1) incorporate lower (10 ng/ml–10 μg/ml) concentrations of protein into a biomimetic calcium phosphate coating applied on titanium alloy (Ti6Al4V); (2) to study the release characteristics of the produced biomimetic coatings and (3) to quantify the amounts of protein both loaded and released. Bovine Serum Albumin (BSA) was chosen as a model protein in this study, because it is inexpensive, has a negative charge at neutral pH (like BMP), and has a molecular weight of the same order as BMP.

Materials and methods
Calcium phosphate coatings were deposited on titanium alloy (Ti6Al4V) by using a biomimetic method as follows: After cleaning, the titanium alloy plates were immersed for 24 hours into a concentrated simulated body fluid under high nucleation conditions. A thin, dense and amorphous calcium phosphate layer was uniformly deposited on the Ti6Al4V surface. The samples were subsequently immersed for 48

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentrations of BSA in CPS (ng/ml)</th>
<th>BSA loading in soaked plates (μg/mg)</th>
<th>BSA loading in the coatings (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>–</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>–</td>
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<tr>
<td>E</td>
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<td>8.1</td>
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<tr>
<td>F</td>
<td>100,000</td>
<td>7.1</td>
<td>16.7</td>
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hours into a Calcium Phosphate Supersaturated (CPS) solution at physiological temperature and pH of 7.4. Various concentrations of Bovine Serum Albumin (BSA) were added into the CPS solution (Table 1).

After immersion, the coatings were characterized by Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Infrared (IR) spectrometry and thickness measurements. The release of BSA from the coatings was investigated by immersing the coated plates in a Simulated Physiologic Solution (SPS) at pH 5 and pH 7.3. Anti-BSA immunological staining clearly showed the incorporation of BSA into the calcium phosphate layer. Enzyme Linked Immunosorbent Assay (ELISA) and Bicinchoninic Acid (BCA) assay measured the amounts of protein loading and release. The results were comparable with those obtained by soaking normal biomimetic coatings in different concentrations of BSA in Phosphate Buffer Saline (PBS) under the same condition as incorporation of BSA in coatings.

Results and discussion

A thick, crystalline coating covered the surface of Ti6Al4V. BSA protein was successfully co-precipitated with the calcium phosphate crystals. With increasing concentrations of BSA protein in CPS solution, the following results were obtained: (1) the coating changed its structure from a highly crystalline octacalcium phosphate (OCP, Ca$_{8}$H$_{2}$(PO$_{4}$)$_{5}$OH$_{2}$) to a poorly crystallised calcium-deficient apatite (Ca$_{10-x}$(PO$_{4}$)$_{6-x}$(HPO$_{4}$)$_{x}$(OH)$_{2}$)$_{n}$; (2) the crystal size decreased indicating a crystal growth inhibition by protein; (3) Amounts of BSA in the coating increased from 0.7 μg/mg to 16 μg/mg; (4) Ca$^{2+}$ release in Simulated Physiologic Solution (SPS) at pH 5 was much slower as compared with coatings produced without BSA (Fig. 2); (5) At higher BSA concentrations, sustained and controlled release of BSA from biomimetic coatings over a 6 days period was demonstrated (Fig. 1).

![Fig. 1. BSA release in SPS from Biomimetic coating at pH 7.3.](image)

![Fig. 2. Controlled Ca$^{2+}$ release from biomimetic coatings in SPS pH 5.](image)
These results indicated that controlled and slow release of protein and calcium from biomimetic calcium phosphate coatings is possible. It allows the production of slow and effective drug release systems for orthopedic applications.

**Conclusions**

From our results we conclude that BSA can be incorporated into biomimetic coatings. We expect biomimetic coatings thus to be able to act as a carrier for proteins in general. A slow dissolution rate of the coatings may lead to a slow protein release. We will apply this method to incorporate growth factors like BMP in the coating, which will help us in producing truly osteoinductive coatings.

**References**


**DESIGN DEVELOPMENT OF A TRANSDERMAL PATCH WITH AN EXPERIMENTAL CNS DRUG**

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**Introduction**

In recent years, the permeation of drugs through the skin has proven to be an important ‘controlled drug delivery’ technique, as judged by the number of transdermal patches introduced to the market. This success has arisen because transdermal drug delivery can provide potential advantages over oral and intravenous therapy including avoidance of ‘first-pass’ gut and hepatic metabolism, fewer side-effects, increased compliance, controlled plasma levels and reduction in overall dosage.

Our research program consists in the development of a ‘systematic approach’ to implement the patch technology with an experimental CNS drug as model drug. The implementation of this approach includes different phases which are discussed below.

**Experimental methods**

**In vitro permeation**

The experiments were performed in modified Franz diffusion cells at 32°C. Using full human skin the experiments were done for 24 hours. The permeation experiments on stratum corneum were performed for 20 days. The receptor phase was 10% HPβCD and 0.05% NaN₃ in water at pH5.

After the experiments the CNS drug was extracted from the skin with dichloromethane. The drug content of the receptor solution and the skin extract was determined using HPLC.

**Preparation of the patches**

The preparation of the patches is performed with a Mathis labcoater type LTE-S. The backing is the Scotchpack 1006 backing of 3 M. The used release liner is the Scotchpack 1022. The patches are prepared with the next process parameters: coating thickness 0.2 mm, coating speed 0.2 m/min, drying time 15 min, drying temperature 45 degrees Celcius and an airflow flows at the surface of the coating.

**In vitro release test**

A modified rotating cylinder method was used for 20–30 days. The receptor phase was 10% HPβCD and 0.05% NaN₃ in water at pH5. Samples were taken at pre-determined time intervals for HPLC analysis.

**Results and discussion**

As a first step different solvent systems and polymers are investigated on the solubility of the drug of interest. Another selection criterion is the compatibility of the drug with both the solvent system and the polymer. This study indicated that benzyl alcohol was the best solvent system. Two polymers, Durotak 87-2100 and Eudragit E100, were selected.

Second, in vitro permeation tests on full human skin and stratum corneum were used to determine the influence of permeation enhancers and to optimise the formulation. The addition of Laurydone, Brij 35 or lauryl pyrrolidone resulted in higher skin accumulation and permeation through stratum corneum. The optimal formulation consists of benzyl alcohol, 5% laurydone and drug at 90% of saturation.

The third step of the approach includes the incorporation of these compounds in the polymer Durotak 87-2100. After the preparation of the polymer-based formulation a patch can be prepared. In vitro release tests were used to determine the release profiles of the patches. Modifying the concentrations of plasticizer and cross-linker can provide the desired release properties. Again in vitro permeation tests the permeation properties of the patches are investigated. These results were used to obtain the optimal drug in adhesive patch.