Equal channel angular pressing (ECAP) as a method applicable to large samples for strengthening metallic materials via severe plastic deformation has recently attracted considerable interest. For biomedical applications, ECAP-treated pure titanium is a promising alternative for implants subjected to high mechanical loads as it contains no potentially cytotoxic alloying elements. The consequences of an ECAP treatment for the physico-chemical properties of the air formed passive layers of pure or alloyed titanium, which are of the utmost importance for the biological response to these materials, are studied here for the first time. This includes mechanical, electron microscopic, and electrochemical investigations in protein containing media to study the reactions that occur immediately after implantation in the phase boundary between implant and biological system. The ECAP treatment results in a positive shift of the flatband potential and a particularly strong increase of the donor density for the native oxide layers. This is associated with increased electronic conductivity of the oxide layers under anodic polarization.

Titanium based materials combine very good corrosion stability with mechanical properties appropriate for many biomedical applications especially for load bearing implants. However, commercially pure (CP) titanium has mechanical properties inferior to α/β-Ti-alloys such as the widely used Ti6Al4V. These alloys are in turn critically discussed because of potential cytotoxic effects of vanadium and aluminum,[1,2] elements that can be found in high concentrations on the material surface and are also released into the surroundings.[3]

It is thus of great interest to improve the mechanical properties of CP titanium, such as ultimate tensile and fatigue strength, to be able to replace the critically discussed titanium alloys in load bearing biomedical applications.[4,5] The use of severe plastic deformation, multi directional forging,[6,7] and especially equal channel angular pressing (ECAP)[5,8–10] to this end offers numerous advantages. ECAP not only allows for rapid treatment of large samples[10] which can be further processed for biomedical applications, but also results in high dislocation and grain boundary densities of the metallic phase.[12] In CP titanium. This stabilizes the air-formed passive layer and thus increases the corrosion stability compared to as received (AR) coarse grained material.[13,14]

In the biomedical area, multiple in vitro and in vivo investigations have shown that protein adsorption is a very early process in the interaction of biomaterial surfaces with biological systems.[15,16] As specific surface properties, that is, for titanium-based materials the properties of the oxide layers, determine the adsorption of and interaction with proteins, they play a key role in the biological response to these materials.

Furthermore, we have shown earlier that the key parameters flatband potential ($\varepsilon_{FB}$) and donor density ($N_D$) of the n-type semiconducting air-formed passive layer vary for different titanium based materials.[19] Additionally, the electronic conductivity of the oxide layer on Ti6Al4V depends on the underlying metallic phase.[20,21]

In this work the consequences of an ECAP treatment for the physico-chemical properties of the air-formed passive layers of titanium-based materials were investigated for the first time. The experiments were performed under conditions relevant for a biomedical use. This included investigations in protein containing media for insight into reactions occurring immediately after implantation in the phase boundary implant/biological system. We were able to show that an ECAP treatment of both CP titanium and Ti6Al4V resulted in a positive shift of the flatband potential of the native oxide layer, and a particularly...
strong increase of their donor density. This was accompanied by an increased electronic conductivity of the oxide layers under anodic polarization.

Microstructural changes of the materials associated with the ECAP treatment are illustrated in Figure 1, that compares SEM images of both materials and STEM images of Ti6Al4V in the AR and ECAP state. Additionally, scanning transmission electron microscope (STEM) energy dispersive X-ray analyses (EDX) distribution images and electron energy loss spectroscopy (EEL) spectra are presented. The grain size of the four materials as derived from the analysis of scanning electron microscope (SEM) images is summarized together with the HV10 hardness data in Table 1.

For titanium the SEM data reveal a strong ECAP-induced reduction of the grain size by a factor of about 7 (11.1 ± 0.3 to 1.55 ± 0.02 μm). This decrease is associated with a significant increase of the hardness by almost 50%. These data are in good agreement with the literature for similar ECAP conditions both in the grain size reduction[22] and hardness increase.[10,23–25] For Ti6Al4V the SEM images indicate no effect of the ECAP treatment on the grain size of the α phase. For the β phase, however, no separate detectable grains are present after the ECAP treatment, a drastic reduction compared to Ti6Al4V AR (grain size 1.46 ± 0.02 μm) that is in agreement with the findings of Semenova et al.[26] It is also possible that the observed effect is at least partially due to a decrease in the amount of β phase as described by Valiev et al.[8]

In contrast, STEM images show a much finer overall microstructure for ECAP-treated Ti6Al4V as compared to the AR sample, indicating that small angle grain boundaries are not resolved by the etching process in this material. This finding is similar to the grain size differences between SEM and transmission electron microscope (TEM) reported for Ti.[22,24,25] The ECAP effect on the hardness of Ti6Al4V amounts to 25% and is thus smaller than for titanium but still significant, which is in good agreement with ref. [27]. A detailed analysis of SEM images from sample center and edge in both the horizontal and vertical direction showed no significant microstructural differences for any of the four investigated materials (Table S2, Supporting Information).

EDX elemental mapping of AR Ti6Al4V and the ECAP-treated samples show large differences in the elemental distribution of V and Al. Ti and O are homogeneously distributed in both AR and ECAP-treated samples, and while Al and V distribution in the ECAP-treated sample are also mostly homogeneous, they are phase-separated in the AR state. In both samples, oxygen is mainly present at the surface and not diffused into the bulk.

Local EEL spectra of the surface oxide layer and the bulk area are obtained from cross-sectionally prepared samples and are used to quantify the native sample oxide surface layer thickness. For this, the intensity of the O–K edge was plotted as a function from the surface to the depth of the metallic phase for

![Figure 1. Comparison of the ECAP-induced changes in the microstructure of Ti and Ti6Al4V. Data of the as received (AR) and ECAP-treated samples are grouped in the left and right column of the figure, respectively. A,B) SEM images of Ti. C,D) Corresponding EEL spectra (from cross-sectioned samples) of the surface oxide layer (blue) and the bulk area of the samples (red). E,F): STEM images of Ti6Al4V. G,H) STEM images and EDX-derived elemental mappings of Ti, O, Al, and V from the regions marked in the STEM images. I,J) Corresponding EEL spectra. The absorption peaks at the L1 and L2 edges are due to excitations from the Ti 2P_{1/2} and 2P_{3/2} core level states into the unoccupied 3d _{t_{2g}} and _{e_{g}} states, respectively.](image-url)
Fitting the resulting intensity at the surface by a Gaussian resulted in oxide layer thickness data of 7 ± 1 and 23 ± 2 nm for Ti in the AR and ECAP state, respectively; for Ti6Al4V these values are 6 ± 1 nm for AR and 21 ± 1 nm for ECAP. These more than threefold thicker oxide layers for ECAP-treated materials are contrary to Garbacz et al.[28,29] who did not find an effect of ECAP treatment of oxide layer thickness. In addition, a strong modification of the Ti-L fine structure was visible due to the altered oxidation state in the surface area (Figure 1). To increase the signal-to-noise ratio, spectra are averaged parallel to the surface. Figure 1 shows the Ti-L edge spectra for the individual samples of the oxide surface layer as well as of the bulk. The absorption peaks at the L3 and L2 edges are due to excitations from the Ti 2p 3/2 and 2p 1/2 core level states into the unoccupied 3d t 2g and e g states, respectively. A strong chemical shift between the two spectra (red and blue curves in Figure 1) reveals a strong oxidation of Ti in the surface layer. This also manifests in the fine structures (i.e., the “sharpening”) of the L3 and L2 peaks.

Flatband potentials and donor densities were derived from a Mott-Schottky analysis of the impedance data in analogy to ref. [19]. The relative dielectric constant was set to 50 based on literature[30,31] and in agreement with earlier work.[19] As no literature data are available for oxide layers on Ti6Al4V, a relative dielectric constant of 50 was also used here.

With the exception of Ti6Al4V ECAP in cell medium, the E_{FB} values as a function of the pH for phosphate buffers (Figure 2A) and the media composition for pH 7.4 (Figure 2B) are in a range between −100 and −900 mV_{AgCl} with the trend in diagram (A) with the assigned text “60 mV per pH” indicates the theoretical slope of E_{FB} per pH curves; *, **, *** for p < 0.05, 0.01, 0.001.

Comparing the effects on AR and ECAP materials of perchlorate electrolyte and cell culture medium to phosphate buffer in Figure 2B, opposing results are found for the different materials. While for AR cell culture medium results in more positive flatband potentials compared to phosphate buffer, for both ECAP materials a ranking in the order of phosphate > perchlorate > cell medium is observed. These differences are all significant for Ti, for Ti6Al4V this is true only for phosphate buffer compared to the two other electrolytes.

The data for the donor densities in Figure 2C,D show an extremely significant effect of the ECAP treatment. Whereas the values for both materials in the AR state are between 2.3 × 10^{20} cm^{-3} (for titanium in cell medium) and 6.1 × 10^{20} cm^{-3} (for Ti6Al4V in phosphate buffer 7.4), donor densities increase by more than two orders of magnitude to 1.8 × 10^{21} - 1.1 × 10^{22} cm^{-3} and 3.6 × 10^{22} - 2.8 × 10^{23} cm^{-3} for Ti ECAP and Ti6Al4V ECAP, respectively.

The donor densities for ECAP-treated Ti6Al4V are at least one order of magnitude higher than the corresponding values for Ti
ECAP over all studied media. Further, for both materials the same ranking of perchlorate > cell medium > phosphate (74) is observed.

No other groups have so far compared the semiconductor properties of air-formed oxide layers on ECAP-treated and AR titanium based materials. Although the flatband potentials in Figure 2A,B, are shifted to more positive values due to the ECAP processing, the values remain negative to the body potential given with 400 to 500 mVAgCl by Velten et al. 33 and thus in the “biologically safe region”. Other groups, however, report redox potentials measured in plasma as being in the range of $-334 \pm 9$ mVAgCl for healthy patients aged below 60 years. 14 These values increase to with age and/or diseases such as diabetes type 2 or atherosclerosis to values above $-280$ mVAgCl. 35,36 Given these data, the $E_{FB}$ values measured for both ECAP-treated materials in phosphate buffer pH 7.4 come close the redox potentials and indicate potentially increased electronic conductivity of the oxide layers under these conditions. Interestingly, the flatband potentials determined in cell medium become more negative after ECAP treatment, which might be related to the cysteine/cysteine and glutathione/glutathione disulfide redox systems present in this medium 34 in combination with the increased electronic conductivity of the oxide layers at potentials positive to the flatband potential.

Although a general increase in the dislocation and grain boundary density is expected as a consequence of an ECAP treatment, 37 little is known about specific values for either CP titanium or Ti6Al4V as a function of the ECAP treatment parameters. 12 For the strong increase in the donor densities of CP titanium or Ti6Al4V as a function of the ECAP treatment medium become more negative after ECAP treatment, which thus in the “biologically safe region”. Other groups, how-ever, report redox potentials measured in plasma as being in the range of $-334 \pm 9$ mVAgCl for healthy patients aged below 60 years. 14 These values increase to with age and/or diseases such as diabetes type 2 or atherosclerosis to values above $-280$ mVAgCl. 35,36 Given these data, the $E_{FB}$ values measured for both ECAP-treated materials in phosphate buffer pH 7.4 come close the redox potentials and indicate potentially increased electronic conductivity of the oxide layers under these conditions. Interestingly, the flatband potentials determined in cell medium become more negative after ECAP treatment, which might be related to the cysteine/cysteine and glutathione/glutathione disulfide redox systems present in this medium 34 in combination with the increased electronic conductivity of the oxide layers at potentials positive to the flatband potential.

Possible (electrochemical) reactions during polarization can be described with:

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$\text{Ti} \rightarrow \text{Ti}^{4+} + 4 e^{-}$ for the formation of titanium ions in the interface metal/oxide

$\text{Ti}^{4+} + 2 \text{O}^{2-} \rightarrow \text{TiO}_2$ and their reaction with oxygen ions formed in the interface oxide/electrolyte via dissociation of water for the oxide formation and with:

$2 \text{H}_2\text{O} \rightarrow \text{O}_2 + 4 \text{H}^+ + 4 e^{-}$ for the formation of oxygen by oxidation of water in the interface oxide/electrolyte due to the electronic conductivity of the oxide layer as side reaction.

After 300 s at the open circuit potential (OCP), galvanostatic polarization with increasing current densities from 0.5 to 5 mA cm$^{-2}$ for 120 s each was applied until a maximum potential of 10 VAgCl was reached (Figure 3A–D). The dotted lines with a slope of 2.5 mC V$^{-1}$ cm$^{-2}$ in these diagrams, assigned to 100% Faraday efficiency (FarEff), indicate the theoretical gain of the potential given the case that all charge transferred through the system is used for oxide formation. 39 Thus, higher potentials at comparable time points of polarization can be assigned to higher Faraday efficiencies for the oxide formation for a given material, corresponding to a lower amount of oxygen formation as a side reaction due to the lower electronic conductivity of the oxide layer. Further, for galvanostatic polarization the time axis in the diagrams can also be considered as a charge density axis.

For phosphate buffer 7.4 (Figure 3A,B) the AR state of both materials results in different shapes of the potential/time (charge density) curves. Whereas a continuously rising curve for current densities $\geq 1$ mA cm$^{-2}$ is observed for Ti AR, after a similar start a voltage maximum is detected for Ti6Al4V AR at the end of the 1.5 mA cm$^{-2}$ polarization interval between 600 and 650 s. This corresponds to a transferred charge density of $\approx 350$ mC cm$^{-2}$. Irrespective of these differences, the Faraday efficiency for the formation of anodic oxide of both AR materials is substantially above the corresponding ECAP materials throughout the whole experiment. These materials show flatter curves that start to rise at higher current densities (2.5 mA cm$^{-2}$), indicative of a higher inherent electronic conductivity of the oxide layers.

For perchlorate electrolyte (Figure 3C,D) the potential/time (charge density) curves for Ti AR and Ti ECAP are in general comparable to those for phosphate buffer. However, the current density at which potentials start to rise continuously (the point where the electronic conductivity of the oxide layer requires the formation of additional oxide to be able to transport all charge) is shifted toward higher values. This shift is to 1.5 instead of 1 mA cm$^{-2}$ for Ti AR and to 2 instead of 1.5 mA cm$^{-2}$ for Ti ECAP, with the slopes for higher current densities being steeper for both materials compared to those in phosphate buffer.

For both Ti6Al4V states a very limited increase of the potential is observed for polarization in perchlorate electrolyte. A voltage maximum similar to the one in phosphate buffer occurs for Ti6Al4V AR at current densities ranging from 2.5 to 4 mA cm$^{-2}$ between individual samples. For Ti6Al4V ECAP potentials stay below 3.5 VAgCl even for the highest current density, intersecting the 2 VAgCl line for current densities between 2.5 and 3 mA cm$^{-2}$. Whereas for titanium the Faraday efficiency for the oxide formation of the AR state is clearly above the ECAP state, no such conclusion can be drawn for Ti6Al4V.

Figure 3E shows a quantification of the required charge densities to reach potentials of 5, 8, and 10 VAgCl as a function of the material treatment (AR or ECAP) and the electrolyte for all investigated samples. A value of 2500 mC cm$^{-2}$ with no error bars in the diagram indicates that the target voltage was not reached for the given combination of material and electrolyte.

For Ti AR the charge densities required to reach potentials of 5, 8, and 10 VAgCl increase continuously in both electrolytes, with the values in phosphate buffer being above those of perchlorate electrolyte. All data stay well below 1000 mC cm$^{-2}$ even for 10 VAgCl. Controversially, the required charge densities for
Ti ECAP increase from about 700 mC cm\(^{-2}\) for 5 V\(_{\text{AgCl}}\) to \(\approx\)1500 and 2000 mC cm\(^{-2}\) for 8 and 10 V\(_{\text{AgCl}}\) respectively, and are comparable between the two media. A similar situation is observed for Ti6Al4V AR in both media, but with a parallel shift to higher required charge densities for the perchlorate electrolyte and a strong increase in the required charge densities between 8 and 10 V\(_{\text{AgCl}}\). While charge densities required for 5 and 8 V\(_{\text{AgCl}}\) are comparable between Ti AR and Ti6Al4V AR this is no longer the case for 10 V\(_{\text{AgCl}}\). This potential is reached for Ti6Al4V AR in phosphate buffer only at a charge density twice as high as the one for Ti AR and not reached at all in perchlorate electrolyte. For the two ECAP-treated materials, higher charge densities are required in both electrolytes to reach given potentials, a difference that is already pronounced for 5 V\(_{\text{AgCl}}\).

We thus can rank the electronic conductivities of the forming anodic oxide layers in the initial phase of polarization in the order of Ti6Al4V ECAP > titanium ECAP > Ti6Al4V AR-titanium AR, which is in perfect agreement with the \(N_d\) data presented in Figure 2C,D.

The increasing slope of the potential/time (charge density) curves for ECAP-treated titanium at potentials > 6 V\(_{\text{AgCl}}\) indicates an increasing Faraday efficiency. Anodic oxide formation on
titanium based materials in neutral electrolytes results in oxide layers from two sub-layers. While the inner part is formed via solid state reaction between titanium ions and oxygen ions in the metal/oxide interface, the outer part is formed via “precipitation” by transferred titanium ions with electrolyte components in the interface oxide/electrolyte. Here we postulate that the growing outer part of these oxide layers is mainly responsible for the increased Faraday efficiency, as its properties will not be affected by the dislocation density in the metallic phase.

In conclusion, we present the first physico-chemical data on the properties of air-formed and anodically prepared oxide layers on ECAP-treated titanium materials. Studying changes in the flatband potentials, donor densities, and electronic conductivities of the oxide layers, we conclude that these properties potentially influence the biological response to such materials via increased redox interactions with adsorbed proteins.

### Experimental Section

Cylindrical samples from CP titanium grade 2 (Ti/titanium) and Ti6Al4V-ELI (Ti6Al4V) (for the chemical analysis of both materials see Table S1, Supporting Information) were ECAP-treated under the following conditions:

| Material | ECAP Passes | Route | Temperature |
|----------|-------------|-------|-------------|
| Ti       | 6           | B120  | 400 °C      |
| Ti6Al4V  | 6           | B120  | 400 °C      |

Afterward samples were metallographically prepared and contacted with a wire made of CP titanium. For TEM investigations, thin lamellas were prepared using a focus ion beam (Dual Beam FIB, FEI Helios 660). The lamellas were further polished using slight Ar ion milling utilizing a Fischione Nanomill model 1040 to remove surface damage stemming from the Ga FIB.

The electrolytes used were 0.133 m phosphate buffer (phosphate) (pH 7.4, adjusted with diluted NH4OH solution) and cell culture medium (cell medium). Cell medium was based on Dulbecco’s modified Eagle medium (DMEM) and contained 10% fetal calf serum.

Microscopic investigations were conducted using a Zeiss DSM 982 Gemini SEM and a JEOL JEM F200 STEM. EELS applied a Gatan ContinuumER spectroscopic image filter, and EDX were obtained from a window-less JEOL dual detector EDX system. Spatially resolved hardness was measured with a testing machine KB-2508VZ.

EPR and N4 data were derived from electrochemical impedance spectroscopy (EIS) measurements in a potential range from −0.5 to +1 V vs. Ag/AgCl and a frequency range from 10 kHz to 1 Hz. Stepwise galvanostatic polarization (GP) with increasing current densities (0.5 to 5 mA cm−2) was run with 120 s polarization time at each current density.

An electrochemical work station VMP3 (software package EC-Lab V 10.34) was used for all EIS and GP measurements. All electrochemical measurements were performed at a temperature of 25 °C.

### Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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### Conflict of Interest

The authors declare no conflict of interest.

### Keywords

donor density, equal channel angular pressing, flatband potential, oxide layer properties, titanium
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