Influence of equal-channel angular pressing on the functional characteristics of biodegradable Fe-based alloys

O V Rybalchenko 1,2*, N Yu Anisimova 2,5, M V Kiselevsky 2,5, G V Rybalchenko 3, N S Martynenko 1,2, N Yu Tabachkova 2,6, N R Bochvar 1, I V Shchetinin 2, A A Tokar 1,2, A G Raab 4 and S V Dobatkin 1,2

1 A A Baikov Institute of Metallurgy and Materials Science of RAS, Leninsky prospect, 49, 119991, Moscow, Russia
2 National University of Science and Technology “MISIS”, Leninsky prospect, 4, 119991, Moscow, Russia
3 P N Lebedev Physical Institute of RAS, Leninsky prospect, 53, 119334 Moscow, Russia
4 Ufa State Aviation Technical University, Institute of Physics of Advanced Materials, K. Marx Street 12, 450000 Ufa, Russia
5 “N. N. Blokhin National Medical Research Centre of Oncology” of the Health Ministry of Russia, Kashirskoe sh. 24, 115478 Moscow, Russia
6 A M Prokhorov General Physics Institute of RAS, Leninsky prospect, 38, 119991 Moscow, Russia
*E-mail: rybalch@mail.ru

Abstract. In this work samples of Fe-Mn alloys were subjected to equal-channel angular pressing (ECAP) in order to study the effect of the resulting microstructure on the mechanical properties, biodegradation rate, and biocompatibility in vitro of the alloys. The microstructure of the alloys was studied by scanning and transmission electron microscopy, as well as by X-ray diffraction analysis. Mechanical properties were studied by microhardness and tensile tests. It is shown that as a result of deformation by the ECAP method, a predominantly austenitic ultrafine-grained (UFG) structure is formed, shear bands up to 600 nm thick and nanosized twins up to 40 nm thick were observed. The release of α-Mn particles in the ECAP process was revealed. Due to the structure refinement and twinning during ECAP, the strength characteristics of Fe-Mn alloys were significantly increased compared to the corresponding alloys in the initial annealed state. An increase in the propensity to biodegradation of UFG alloys with their satisfactory hemocompatibility was observed.

1. Introduction
The development of biodegradable iron-based alloys for temporary medical implants has been the subject of intensive research during recent years [1], [2], [3]. Biodegradable implants do not require additional surgery to remove the implant, potentially reducing long-term risks and side effects [4], [5], [6]. The suitability of iron for using as a biodegradable implant material has been shown in vivo studies of pure iron stents [4], [6], [7]. However, the rate of degradation of pure iron in vivo is too low and rather close to the properties of materials for permanent implants [4], [6].

The number of non-toxic alloying elements that can be doped with Fe when creating biodegradable medical alloys is very limited. Currently, Fe-Mn alloys with a Mn content of more than 25 wt. % are
considered acceptable for use as a material for the manufacture of biodegradable implants, since they are characterized by increased degradation rates [8], [9], [10]. The manganese content in such alloys allows to obtain a non-magnetic state, which makes possible to use magnetic resonance imaging as a method of monitoring the implanted product during operation [11].

Nevertheless, the issue of controlling and increasing the biocorrosion rate of Fe-Mn alloys remains open. The ability to control the rate of corrosion of biodegradable metals will make it possible to match the mass loss with the release of corrosion products during in vivo biodegradation and the limit of tolerance of these products to surrounding tissues, i.e. biocompatibility [12]. Therefore, the control of the processes of biodegradation using the refinement of the structure up to ultrafine-grained without changing the chemical composition of the main alloy is considered promising idea. It is assumed that, equal-channel angular pressing (ECAP) can be an excellent opportunity to control the rate of biocorrosion by regulating the structural – phase state by varying ECAP modes.

However, it is not yet clear that the ultrafine-grained structure increases the rate of corrosion. Thus, in [13], ECAP increased the corrosion resistance of iron in the ultra-fine grained state with a grain size in the range of 80-200 nm compared to the coarse-grained state with a grain size over 50 microns. In the case of magnesium alloys it is reported that RCAP has an ambiguous effect on corrosion resistance, both improving it together with improving biocompatibility [14] and worsening it [15].

This behavior is caused not only by grain refinement during the ECAP process, but also by changes in the phase composition of the material. Usually, the presence of a phase with significant deviation of corrosion resistance from the average one in the structure of a material leads to the appearance of an electrogalvanic pair, which most often accelerates the degradation process [15]. Therefore, in order to control the rate of degradation during ECAP, it is important to monitor not only the grain size, but also the presence of additional phases.

In this paper, we study the possibility of creating a structural-phase state of Fe-Mn alloys during ECAP, which improve the complex of chemical and biological characteristics. The obtained characteristics combined with increased mechanical properties make the material potentially attractive for use as the biodegradable implants.

2. Experimental

Two Fe-Mn alloys of nominal composition Fe-30 wt% Mn and Fe-45 wt% Mn were melted in the Leybold Heraeus Vacuum Arc Remelting (VAR) furnace L200DI. The ingot was remelted up to ten times and then hot forged at 1100 °C into round bars to a diameter of 12 mm. The alloys were then annealed at 1100 °C. This state served as basis for the subsequent severe plastic deformation by method of equal-channel angular pressing (ECAP). ECAP was carried out via route Bc on billets of 10 mm in diameter and 60 mm long using a die with channels intersecting at 120° with a graphite lubricant [16].

For maximum structure refining the samples of Fe-30Mn alloy were processed by six ECAP-passes and just by three ECAP-passes for Fe-45Mn alloy at 450 °C. A high deformation temperature of 450 °C was chosen to suppress the martensitic transformation in Fe-Mn alloys during ECAP. The true strain applied to the billet per pass for the defined die geometry equals 0.9 (shear strain \( \gamma = 1.5 \)) [17, 18].

The microstructure of the experimental specimens was observed by scanning electron microscopy (SEM) by JSM-7001F (JEOL) after etching with a 3% HNO₃/alcohol solution. The microstructure was investigated using a JEM-2100 transmission electron microscope operated at 200 kV. X-ray diffraction (XRD) (Rigaku Ultima IV diffractometer) using Co Kα radiation was employed to identify the constituent phases of the Fe–Mn binary alloys.

The tensile tests were performed at room temperature using an INSTRON 3380 machine with a strain rate 1 mm/min and a load capacity of 100 kN.

In order to study the biocompatibility of the alloy we have evaluated the level of hemolysis and cell toxicity. Cells isolated from the blood of intact C57BL/6 mice were used for research. The alloys were considered to be biocompatible when the level of induced hemolysis did not exceed 2%, and the cell viability did not significantly differ from the control. A detailed method for assessing the level of hemolysis and cytotoxicity is given in [19].
The degradation rate was studied on alloy samples with the size about one quarter of a disk with a diameter of 10 mm and a thickness of 1.5 mm. We used at least 3 samples per point. The study was conducted at 37 °C in two corrosive media (foetal bovine serum (FBS) and RPMI-1640 (both PanEco, Russia)) within 7 and 30 days. Cleaning the surface of the samples from degradation products as well as calculating the degradation rate was performed in accordance with ASTM_G1-03-E.

The cell and animal test protocols used in this work were evaluated and approved by the local Ethics Committee of N.N. Blokhin NMRCO.

3. Results and Discussion

On the figure 1 SEM micrographs showing the microstructure of the Fe–30%Mn binary alloy (figure 1a) and the Fe–45%Mn alloy (figure 1b) after forging with the average grain sizes about 80 µm and 50 µm, correspondingly, are presented. The X-ray analysis after forging revealed the presence of a fully austenitic structure in an alloy with 30 wt% Mn and a multiphase structure in an alloy with 45 wt% Mn, consisting of 95.2% of \( \gamma \)-phase, 2% of \( \alpha \)-phase and 2.8% of \( \varepsilon \)-martensite.

![Figure 1. SEM micrographs of the microstructure of Fe-30%Mn (a), Fe-45%Mn alloys after forging](image)

Subsequent homogenization results in a completely austenitic state of the Fe-45% Mn alloy. In general, long term high temperature annealing results in diffusion processes that eliminate chemical and structural inhomogeneity, that is especially important for alloys with Mn content. The grain growth during the annealing with an attendant decrease in the yield strength makes the material more pliable for applying severe plastic deformation using the ECAP method.

ECAP of the Fe-30% Mn alloy results in the formation of an ultra-fine grained structure within shear bands from 100 to 600 nm thick. The shear bands are intersected by thick dislocation cross bulkheads and twins with a thickness of 34.4 ± 4.1 nm. As a result, a subgrain structure with the size of structural elements up to 100 nm (figure 2 a, b) is formed. In the Fe-45% Mn alloy samples, an ultrafine - grained structure is also formed after ECAP by cutting of shear bands with a thickness of 100-400 nm by thick dislocation cross bulkheads and nanotwins with a thickness of 20.0 ± 2.3 nm (figure 2 c). It was noted that in this case particles from 10 to 40 nm in size are quite common (figure 2 d). According to the FFT (Fast Fourier Transform) pattern from an individual particle shown in the insert to figure 2 d, the interplanar spacings of the obtained phase corresponds to \( \alpha \)-Mn.
ECAP significantly increases strength characteristics of the Fe-30% Mn alloy compared to the forged state ($\sigma_{UTS} = 1515$ MPa; $\sigma_{YS} = 1327$ MPa and $\sigma_{UTS} = 960$ MPa; $\sigma_{YS} = 587$ MPa for ECAP-treated and forged states, respectively) with satisfactory ductility (12.3% compared to 42.7% in the forged state). The failure of the test samples with 45% Mn under tension occurs in the elastic region. Despite the fact that the volume fraction of $\alpha$–Mn particles in the Fe-45% Mn alloy after ECAP is small and cannot be determined by the X-ray diffraction, it is the brittleness of $\alpha$–Mn that can explain the lack of plasticity in 45% Mn alloy samples. At the same time, the microhardness of alloys after ECAP equally increases from 2.32 ± 0.05 GPa to 5.43 ± 0.13 GPa and from 2.67 ± 0.34 GPa to 6.61 ± 0.30 GPa for Fe-30% Mn and Fe-45% Mn alloys, respectively.

The results of the in vitro biocompatibility study of the Fe-Mn alloys are shown in figure 5. Studies of red blood cells (RBC) hemolysis have shown that both studied alloys in both microstructural states (in the initial annealed state and after ECAP) are hemocompatible, since the level of induced hemolysis of samples does not exceed 5% and they do not have a statistically confirmed cytotoxic effect on blood cells (figure 5 a). Despite the fact that the hemolytic activity of the Fe-45%Mn alloy increased to 7% after ECAP, this sample can also be considered hemocompatible, since its difference from other alloys in the test for hemolytic activity was not statistically verified (P>0.05).
At the same time, the study of the cytotoxicity of alloys showed no apparent cytotoxic effect of alloys on white blood cells (WBC) (figure 5 b). The cytotoxicity of both alloys in both microstructural states does not exceed 0.5% and does not statistically differ from the level of cytotoxicity in the control. A slight deviation from the control is observed only for the annealed Fe-45% Mn alloy, but the difference is negligible. It is also important to note that ECAP of both studied alloys, in general, does not increase their hemolytic and cytotoxic activity in relation to the annealed state. The only exception is the Fe-45% Mn alloy, for which the cytotoxicity after ECAP slightly decreased.

Studies of the biodegradation of alloys in the annealed state and after ECAP did not reveal a significant increase in the rate of degradation after deformation, but revealed an increase in biodegradation of alloys after severe plastic deformation during incubation of samples in FBS for a week (figure 6). Such an increase for deformed alloys may be explained by the formation of a large density of lattice defects, primarily dislocations, during the ECAP process, as well as a predominantly subgrain structure with a large number of shear bands. In this case, incubation of alloy samples for a month practically does not reveal differences in the rate of degradation of annealed and deformed samples. At the same time, the increase in the incubation period from 7 to 30 days, in general, led to a decrease in the average rate of degradation. Apparently, an increase in the duration of incubation leads to the formation of a layer on the samples surface consisting of corrosion products of alloys. This layer partially protects the sample surface and decrease degradation rate thereby eliminates the effect of severe plastic deformation in the case of the ECAP-treated alloys, slowing down the corrosion process [14].

**Figure 3.** Mechanical properties of Fe-Mn alloys after forging and ECAP

**Figure 4.** Microhardness of the Fe-Mn alloys after annealing and ECAP
4. Conclusions
The study considers the ultrafine-grained Fe-30% Mn alloy as a promising material for medical use. It has been shown that ECAP at $T = 400$ °C significantly increases the strength characteristics of Fe-30% Mn alloy without deteriorating its biocompatibility in vitro. The high specific strength of the obtained material will make it possible in the future to create a miniature implantable product, the biodegradation of which will take less time. At the same time, incubation of samples in the culture medium for a week revealed an increase in the corrosion rate in deformed samples. Longer incubation in biological environment does not reveal a similar effect as a result of surface protection by corrosion products from further degradation.

The lack of ductility of Fe-45% Mn alloy after ECAP does not allow us to recommend this alloy for medical use now. However, high rate of degradation in the short term and good biocompatibility in vitro, make this alloy attractive for farther improvement by another ECAP regimes.

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