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Proteins and medium-flow conditions: how they influence the degradation of magnesium

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To understand the magnesium (Mg) degradation mechanism *in vivo*, a variety of *in vitro* investigations have been conducted. However, there is still a gap between *in vitro* results and *in vivo* observations. Herein, the combined effect of proteins and medium-flow conditions on magnesium degradation was studied. Bovine serum albumin (BSA) and fetal bovine serum (FBS) were employed as the commonly used protein components for *in vitro* tests. Static, medium-changing (semi-static) and continuous-medium-flow (dynamic) conditions were used as different conditions. The results showed the highest inhibition efficiency of BSA and FBS on magnesium degradation under static conditions, while the efficiency largely decreased under semi-static or dynamic conditions, particularly for BSA. The possible explanations are related to the composition of degradation products and the integrity of the degradation layer. These results emphasize the importance of considering effects from different factors during magnesium degradation.

Notation

A	surface area exposed to the medium
$DR_{\text{with protein}}$	degradation rate of magnesium (Mg) in the protein-containing medium
$DR_{\text{without protein}}$	degradation rate of magnesium in the medium without protein
m_1	weight of the sample before immersion
m_2	weight of the sample after the removal of degradation products
t	immersion time
ρ	density of pure magnesium

1. Introduction

Magnesium (Mg) and its alloys have gained increasing attention as metallic biomaterials due to their good biodegradability and compatibility.^{1–3} However, there is still a large gap between the *in vitro* and *in vivo* results for magnesium degradation due to the complex environment under *in vivo* conditions.^{4–6} To fill this gap and lay a solid foundation for the design and development of magnesium-based materials, it is necessary to gain a deeper understanding of the *in vivo* degradation mechanism of magnesium-based biomaterials. This, however, is quite difficult because the degradation *in vivo* cannot be monitored at all time points and with high spatial, chemical and temporal resolution. Thus, how parameters, which play most likely a role in *in vivo* degradation, influence the process should be systematically investigated.

To achieve this goal, degradation in different solutions has been studied: from simple salt solutions to more complex cell culture media.^{7–9} The effects of various physiological parameters, such as buffering,^{10,11} medium inorganic composition,^{10,12} organic molecules^{13–16} and stress loading,^{17,18} have been investigated. In comparison with the results obtained under *in vivo* conditions,

Earle's balanced salt solution and simulated body fluid have been recommended as proper testing solutions for magnesium degradation in terms of degradation rate.^{7,8} However, organic molecules in the medium have been found to promote the formation of calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) on the magnesium surface and modify the composition of degradation products.^{14,19} These degradation products play an important role in some biological processes – for instance, cell adhesion and surface biomineralization.²⁰ In sum, it is obvious that very complex chemical and biological reactions take place during the degradation *in vivo*, but the understanding about the process is still not sufficient.

Among organic molecules, proteins are the most abundant and the major organic components in human plasma. Their influence on magnesium degradation represented by bovine serum albumin (BSA) and fetal bovine serum (FBS) has been widely studied in different solutions under different conditions.^{21–23} In general, their influence is mainly ascribed to protein adsorption on the magnesium surface and/or the binding/chelating to released ions.^{15,24,25} The adsorption of proteins can decrease the degradation rate of magnesium due to the barrier effect of the adsorbed protein film, while the binding/chelating effect of proteins results in the accelerated degradation of magnesium because of the interference with the formation of degradation products, which leads to an alloy- and medium-dependent influence of proteins on magnesium degradation.^{24,26}

Another factor that influences degradation is the availability of fresh medium to avoid saturation effects. This is realized in the body by constant transport phenomena but often not considered in *in vitro* experiments. To realize the dynamic metabolic equilibrium in the human body, different testing methods for degradable magnesium have been developed, such as simple static immersion in a certain solution volume during the whole testing period, partially or entirely

changing the solution after certain immersion intervals (semi-static)^{8,19,27} and more complex medium-flow conditions by way of a bioreactor system (dynamic).^{28,29} However, the synergistic effect of different parameters on magnesium degradation so far has not achieved enough attention – for example, the comprehensive influence of proteins and the medium-flow conditions.

Therefore, in the present study, the interplay between proteins (BSA and FBS) and three different conditions (static, semi-static and dynamic conditions) on magnesium degradation was investigated.

2. Materials and methods

2.1 Materials

The pure magnesium used in this study was cut into small disks with dimensions of 10 mm × 10 mm × 4 mm from a cast magnesium ingot (purity: 99.94 wt.%, Magnesium Elektron, Manchester, UK), the detailed composition and the microstructure of the material have been presented in a previous report.¹³ Before the immersion test, the surface of each of the pure magnesium samples was ground successively using 800, 1200 and 1500 grid silicon carbide (SiC) abrasive paper (Schmitz-Metallographie GmbH, Herzogenrath, Germany) and then ultrasonically cleaned in *n*-hexane (C₆H₁₄), acetone (C₃H₆O) and 100% ethanol (C₂H₅OH) (Merck KGaA, Darmstadt, Germany) for 20 min. To avoid contamination and maintain sterilization, the samples were ultrasonically immersed in 70% ethanol for 20 min and finally dried under sterilized conditions prior to use.

2.2 Immersion tests

The cell culture medium Dulbecco's modified Eagle medium GlutaMax-I (DMEM) (Life Technologies, Darmstadt, Germany) was used as the base medium for the immersion tests due to the similar degradation of magnesium in it to that under *in vivo* conditions.¹⁹ To prepare the protein-containing medium, 3.4 mg/ml BSA and 10% FBS were added in DMEM, since they have similar protein concentration.¹⁹ Thus, three different media, DMEM, DMEM + 3.4 mg/ml BSA and DMEM + 10% FBS, were used for the immersion tests.

Static, semi-static and dynamic conditions (Figure 1) were the different medium-flow conditions used for the immersion test under

cell culture conditions (37°C, 5% carbon dioxide (CO₂), 20% oxygen (O₂), 95% relative humidity). Each sample was immersed in 20 ml medium for 14 d of immersion; thus, the ratio of the medium volume to the sample surface (V/S) was around 5.88, which refers to the static condition. For the semi-static condition, 3.5 ml medium was used for each sample (V/S = 1.03) and the medium was refreshed after 3, 5, 7, 10 and 12 d of immersion. Therefore, the volume of the medium used for each sample was 21 ml for the semi-static condition. To present a medium-flow condition, a bioreactor was supplied with 20 ml unrenewable medium per sample (V/S = 5.88) during the test at a medium flow rate of 2 ml/min in the tube (dynamic condition). After certain immersion times under different conditions, the pH (Sentron Argus X pH meter, Fisher Scientific, Schwerte, Germany) and osmolality (Osmomat 030, Gonotec, Berlin, Germany) of the medium were measured. For each condition or medium, four replicates were used; three of them were used to calculate the degradation rate of samples by way of weight loss measurement, and the one was used to characterize the surface degradation products.

2.3 Degradation rate

The degradation rates of magnesium under different conditions were determined by weight-loss measurement. The weight of each sample (m_1 in grams) was determined before immersion. After 14 d of immersion, the degradation products on the magnesium surface were removed by immersing in 180 g/l chromium trioxide (CrO₃) in distilled water (VWR International, Darmstadt, Germany) for 20 min. Subsequently, the sample was cleaned with distilled water and ethanol (Merck KGaA, Darmstadt, Germany). After drying, the weight of the sample was determined again (m_2 in grams). The degradation rate of magnesium (DR in millimeters/year) can be calculated by using the following equation

$$1. \quad DR = \frac{8.76 \times 10^4 (m_1 - m_2)}{A \rho t}$$

where A is the surface area exposed to the medium (cm²); ρ is the density of pure magnesium (1.74 g/cm³); and t is the immersion time (h). Furthermore, the inhibition efficiency (IE in percent) of proteins to the degradation of magnesium can be calculated as follows

Figure 1. Schematic illustration of the three different immersion conditions used

$$2. \quad IE = \frac{DR_{\text{without protein}} - DR_{\text{with protein}}}{DR_{\text{without protein}}} \times 100 \%$$

Herein, $DR_{\text{without protein}}$ is the degradation rate of magnesium in the medium without proteins (DMEM) and $DR_{\text{with protein}}$ is the degradation rate of magnesium in the protein-containing medium (DMEM + BSA or DMEM + FBS).

2.4 Surface characterization

After immersion, sample surfaces were analyzed by scanning electron microscopy (SEM; Phenom-World, Eindhoven, the Netherlands) at 15 kV acceleration voltage in backscattering mode. The composition of surface degradation products was identified by point analysis of X-ray energy-dispersive spectroscopy (EDS). At least five points were analyzed for the composition of surface products. Infrared (IR) spectra of the sample surface were acquired by taking 512 scans at a resolution of 2 cm^{-1} in reflectance mode (Bruker Hyperion 2000, Ettlingen, Germany). The Bruker Opus version 7.5.18 software was used to analyze the IR results.

The samples after immersion were embedded in resin to prepare cross-sections of the degradation layer. After successive grinding with 800, 1500 and 2400 grid abrasive paper and polishing with colloidal silica (SiO_2) suspension (Cloeren Technology GmbH, Wegberg, Germany), the thickness of the degradation layer was evaluated from SEM images of the cross-section (Tescan Vega3 SB, Brno, Czech Republic). The distribution of elements in the degradation layer was further analyzed by EDS mapping, which was performed at 15 kV acceleration voltage at a resolution of 256 pixels. The acquiring time was 80 ms per pixel. The thickness of the degradation layer was taken from the cross-sectional SEM images with at least 80 points for each condition.

2.5 Statistical analysis

Statistical analysis of the degradation rate and layer thickness was carried out by using one-way analysis of variance on ranks with Dunn's multiple-comparison post hoc tests. Figures 1, 5 and 7 were organized in the Adobe Photoshop CS5 software (Adobe Systems Incorporated, San Jose, USA); the others were created in the OriginPro 8 software (OriginLab Corporation, Wellesley Hills, USA).

3. Results

3.1 Degradation rate, pH and osmolality

Figure 2 shows the degradation rate of pure magnesium in DMEM, DMEM + BSA and DMEM + FBS under different conditions. The results revealed that the highest degradation rate of pure magnesium was in DMEM under static immersion ($1.358 \pm 0.068 \text{ mm/year}$). Under this condition, the addition of BSA and FBS significantly reduced the degradation rate of pure magnesium to 0.444 ± 0.052 and $0.188 \pm 0.001 \text{ mm/year}$, respectively. Obviously, the semi-static condition ($0.301 \pm$

Figure 2. Degradation rate of pure magnesium in the DMEM-based medium under different conditions. Significance level: * $p < 0.05$, ** $p < 0.01$

0.017 mm/year) and dynamic condition ($0.222 \pm 0.028 \text{ mm/year}$) also presented lower degradation rates for pure magnesium in DMEM than the static condition. The presence of BSA under the semi-static condition resulted in a degradation rate for pure magnesium comparable with that in DMEM, while it led to a much faster degradation ($0.849 \pm 0.035 \text{ mm/year}$) under the dynamic condition. More importantly, the addition of FBS always decreased the degradation rate and a relatively stable degradation rate ($\sim 0.180 \text{ mm/year}$) was determined in DMEM + 10% FBS irrespective of which condition was used.

Furthermore, the inhibition efficiency of proteins on magnesium degradation under different conditions was calculated based on Equation 2, as shown in Figure 3. The figure shows the higher inhibition efficiency of FBS than that of BSA regardless of the

Figure 3. Inhibition efficiency of proteins (BSA or FBS) on magnesium degradation

used condition. Moreover, positive values were determined for FBS irrespective of conditions, while BSA presented negative values for semi-static and dynamic conditions, indicating that FBS was more efficient than BSA for preventing magnesium degradation. However, the most conspicuous observation is that the inhibition efficiency for both BSA and FBS decreased in the following order: static condition > semi-static condition > dynamic condition. This suggests that the medium-flow condition can affect the interaction between proteins and the magnesium surface.

The variations in the pH and osmolality of the medium during immersion under different conditions are shown in Figure 4. The results showed that the pH was always lower than 8.5, indicating the good buffering capacity of the carbon dioxide/bicarbonate (HCO_3^-) system used in this study. Under the static condition, both the pH and osmolality of the medium gradually increased, but the fastest increase was observed in DMEM, showing a good agreement with the degradation rate (Figure 2). For the semi-static condition, the pH first showed a high value and then gradually decreased or remained stable. A similar variation could be found for osmolality. This was caused by the low volume of the medium used, the refreshing of the medium every 2 or 3 d and the decreasing degradation rate of magnesium with the immersion time.³⁰ In the case of the dynamic condition, the variations in pH and osmolality were similar to that under the static condition, but with a relatively fast increase for osmolality, particularly for the initial several days, suggesting the fast diffusion process under the dynamic condition.

3.2 Top-view degradation morphology and degradation products

The top-view surface morphologies of pure magnesium in different media under different conditions are shown in Figure 5. The cracks during the drying process are visible for all samples. Some holes were observed on the degradation product layers formed in DMEM under the static condition, which was caused by the peeling off of degradation products due to hydrogen (H_2) evolution during immersion. Except for the defects existing in the degradation product layer (indicated by the arrows in Figure 5) formed in DMEM under static immersion and in DMEM + BSA under static and dynamic immersion, few differences were found.

EDS was performed to characterize the composition of degradation products on pure magnesium, as listed in Table 1. In general, the products were composed of magnesium, oxygen (O), phosphorus (P), calcium (Ca), carbon (C) and sodium (Na). The existence of sodium was caused by the remained composition of medium after immersion. Oxygen and magnesium are the major elements of the degradation products due to the formation of magnesium-based products, such as magnesium hydroxide ($\text{Mg}(\text{OH})_2$) and magnesium carbonate (MgCO_3).³¹ The presence of calcium resulted from the formation of calcium-involved insoluble products, which were derived from calcium ions (Ca^{2+}) in the testing medium. The percentages of phosphorus and calcium in degradation products were evidently different, which generally presented higher contents in medium containing BSA or FBS, except under static condition. This is in accordance with the

Figure 4. Variations in pH and osmolality during immersion in different media under different conditions

Figure 5. Top-view SEM images of samples after immersion in different media under different conditions (the arrows point to the defects of the degradation product film on samples)

authors' previous report^{13,19} that organic molecules promote the formation of calcium–phosphorus salts on the magnesium surface under cell culture conditions.

The degradation products were further characterized by using IR spectra. The typical spectra are shown in Figure 6. The very broad band in the range $3600\text{--}2700\text{ cm}^{-1}$ is caused by water, which always exists on hydrophilic surfaces due to the strong hydrogen (H) bonds of water. The band around 1640 cm^{-1} is ascribed to the OH bending of absorbed water and/or the amide I of organic molecules absorbed on the magnesium surface.^{19,32} The broad band from 1600 to 1300 cm^{-1} should be a result of the stretching of the carboxyl bond in organic molecules and carbonates.^{23,33} The following broad band at 1025 cm^{-1} is attributed to the stretching of the phosphate (PO_4^{3-}) bond,³⁴ and the bending of the carbonate bond resulted in the bands at 859 and 579 cm^{-1} .¹³ The weak band near 3700 cm^{-1} is an indication of the existence of magnesium hydroxide.³⁵ Similar products on the magnesium surface in different media can be concluded as carbonates, phosphates, adsorbed organic molecules and magnesium hydroxide, which fit well with the similar pH for all samples after the immersion. Comparing the intensities of different bands, it can

be seen that the addition of BSA or FBS largely enhanced the band intensity from phosphates, which agrees with preceding

Figure 6. IR results for samples in DMEM-based media under semi-static conditions

Table 1. Weight percentages of elements in the degradation products of samples under different conditions

Conditions	Medium	Oxygen: wt. %	Magnesium: wt. %	Phosphorus: wt. %	Carbon: wt. %	Calcium: wt. %	Sodium: wt. %
Static	DMEM	53.0	12.7	11.1	8.2	14.1	0.9
	DMEM + BSA	54.6	16.5	9.2	8.7	9.8	1.2
	DMEM + FBS	51.8	15.2	13.2	7.8	11.2	0.8
Semi-static	DMEM	59.8	26.2	1.6	9.0	2.5	0.9
	DMEM + BSA	54.3	16.0	11.4	8.2	9.3	0.8
	DMEM + FBS	50.6	11.2	14.3	8.2	15.0	0.7
Dynamic	DMEM	57.2	22.7	6.3	8.7	4.1	1.0
	DMEM + BSA	52.0	14.6	12.4	8.0	12.1	0.9
	DMEM + FBS	52.7	12.9	13.1	7.7	12.9	0.7

Figure 7. EDS element mapping of the representative cross-sectional degradation layer formed under different conditions

EDS result that proteins promote the formation of calcium–phosphorus products on the magnesium surface.

3.3 Cross-sectional degradation morphology and layer elements

To present the elemental distributions in the degradation layer, EDS mapping was conducted on the cross-sections of the degradation layer, as shown in Figure 7. Due to the low amount of sodium as listed in Table 1, it is not presented in Figure 7. The results showed that carbon was mainly distributed in resin, while oxygen and magnesium were abundant in the degradation layer. Calcium and phosphorus were mainly located in the top of the degradation layer, which results in the formation of a calcium/

phosphorus-rich top degradation layer no matter which condition or medium was used. Combined with IR results, this suggests the formation of calcium–phosphorus salts in the top of the magnesium surface. Furthermore, higher contents of phosphorus and calcium and a thicker top calcium/phosphorus-rich layer could be found in DMEM + BSA/FBS than in DMEM, particularly under semi-static and dynamic conditions. It should be noted that an obvious penetration of resin into the degradation layer could be found for the sample immersed in DMEM under static immersion and the sample in DMEM + BSA under the dynamic condition, indicating the breakage of the degradation layer, which is in agreement with the SEM observation (Figure 5).

Figure 8. Thickness of the degradation layer formed in different media under different conditions (at least 80 points were taken) and the corresponding degradation rate of magnesium (The boxes (bottom to top) show the 25th percentile, median and 75th percentile. The whiskers mark the tenth and 90th percentiles; the rectangular points in the box are average values. Significance levels: * $p < 0.05$, ** $p < 0.01$)

The thickness of the degradation layer in different media under different conditions was measured according to the cross-sections of the degradation layer, which is shown in Figure 8. It reveals the thickest degradation layer in DMEM under static immersion, while the thinnest one in DMEM + FBS under the dynamic condition, which fits well with the degradation rate of pure magnesium. The presence of BSA or FBS in the medium led to the formation of a thinner degradation layer, except for BSA under the dynamic condition. Comparing with the degradation rate of pure magnesium, it can be found that a higher degradation rate corresponds to a thicker degradation layer.

4. Discussion

To obtain more in vivo-like degradation, many researchers have always tried to control the pH of the testing medium within the physiological range (7.2–7.4) by way of different buffering systems or medium-flow set-ups.^{11,36,37} In this study, a carbon dioxide/bicarbonate system was used as the buffering system, and the pH of the medium under different conditions was in the range 7.7–8.3 (Figure 4), indicating proper buffering used during immersion. Besides the buffering capacity, the introduction of carbon dioxide into the immersion system also affected the composition of degradation products, which promoted the formation of carbonate on the magnesium surface (Figure 6). This in turn contributed to the degradation rate and the pH increase.^{38,39} Moreover, the existence of calcium ions in the testing solution, combined with the presence of bicarbonate and hydrogen phosphate (HPO_4^{2-}) ions, is proven to largely inhibit the increase in pH and the degradation of magnesium by way of the enhanced protective properties of degradation products on the surface.^{40,41} Thus, the carbon dioxide/bicarbonate system and the proper medium used in this study guaranteed the suitable pH range for magnesium degradation

during the test. All immersion tests, static, semi-static and dynamic, showed an initial increase in pH (Figure 4), which is a typical behavior of magnesium degradation due to the attack of fluid on the bare magnesium surface, which is in agreement with the report result.⁴² During the following immersion, the pH of the medium reached a relatively stable level (7.7–8.3) (Figure 4), except for the fluctuation of pH under the semi-static condition when the medium was refreshed.^{33,43} The general lower pH value in the medium containing proteins (except DMEM + BSA under the dynamic condition) was caused by not only the slower degradation rate of magnesium but also the buffering capacity of proteins (carboxyl and amino groups).

The results from the static immersion test showed the highest inhibition efficiency of proteins on magnesium degradation (Figure 3). When magnesium is immersed in the medium, magnesium dissolution occurs and leads to the release of magnesium ions (Mg^{2+}) and an increase in pH. The reduction of oxygen is also possible for magnesium degradation as the cathodic reaction, leading to the less dissolved oxygen in the medium.⁴⁴ Subsequently, depending on the product solubility, degradation products, such as magnesium/calcium- PO_4 , magnesium/calcium- CO_3 and magnesium hydroxide, are formed on the surface (Figures 5 and 6), which in turn reduces the magnesium dissolution due to the barrier effect. In static immersion, the equilibrium between magnesium degradation and the formation of degradation products could be reached as the immersion is prolonged, as indicated by the stable degradation rate after long-term immersion.^{10,30} As reported in the literature,^{21,25,45} proteins can adsorb on the magnesium surface during immersion and then prevent the penetration of aggressive ions – for example, chloride ions (Cl^-). Thus, this can decrease the degradation rate of magnesium. On the other hand, the binding/chelating of proteins to magnesium ions can increase the degradation rate of magnesium due to its disturbance in the formation of degradation products.^{15,22,24,46} However, in this study, the addition of proteins in static immersion seemed to promote the formation of a calcium/phosphorus-rich top layer (Figure 7), which is believed to inhibit the degradation of magnesium.¹⁹ Since lower pH was determined in DMEM + BSA/FBS than in DMEM (Figure 4), it can be stated that this promoted calcium-phosphorus salt formation was probably not caused by the supersaturation of calcium-phosphorus salts at higher pH but was induced by adsorbed proteins due to the attraction of organic groups to calcium/phosphate ions.²⁰ Thus, the adsorption of proteins and the induced formation of calcium-phosphorus products play predominant roles in magnesium degradation in a protein-containing medium under static conditions; eventually the presence of proteins leads to slower degradation of magnesium.

Semi-static immersion has often been used to predict the degradation of magnesium in vivo,^{8,12,27} which is believed to be more ‘realistic’ than static immersion, since the immersion medium is refreshed after certain intervals. Ensuring that the same medium volume was used during immersion resulted in a lower

V/S for semi-static immersion (1.03) than for static condition (5.88), which easily led to the accumulation of magnesium and hydroxide (OH^-) ions in the medium as reflected by the higher osmolality and slightly higher pH after 3 d of immersion (Figure 4). However, the degradation mechanism of pure magnesium was still the same as that under the static condition (Figure 5). When the medium was refreshed, the alteration of the environment surrounding the sample occurred suddenly, which re-promoted the degradation of magnesium due to the lower pH and osmolality presented and the possible dissolution of precipitated products. Thus, the degradation of pure magnesium should be gradual under the semi-static condition as the medium was changed, while the degradation rate still showed a decreasing trend from the point of view of long-term immersion.^{30,42} In this case, protein adsorption had occurred on the magnesium surface before the medium change, which further impeded the penetration of the refreshed medium after the medium change, thereby leading to the inhibition of proteins on magnesium degradation (Figures 7 and 8).¹⁹ It should be noted that a lower inhibition efficiency of proteins was found under semi-static conditions compared with that under static immersion. This is a comprehensive result of many factors, such as S/V ratio, testing duration and medium change intervals.

The introduction of flow into the immersion test enables investigation of the comprehensive effect of proteins and dynamic conditions on magnesium degradation. In general, fluid flow is believed to promote the degradation rate of magnesium due to the accelerated diffusion process and the induced shear stress.^{28,29,47} However, in this study, the degradation rate of pure magnesium in DMEM, DMEM + BSA and DMEM + FBS under dynamic conditions was slower than, faster than or similar to that under static immersion (Figure 2), suggesting the complex comprehensive effect of fluid flow and proteins on magnesium degradation. It has been reported that the flow condition promotes protein adsorption and seems to increase the extent of protein adsorption on the surface.^{48–50} However, all of these studies were performed on a stable (unchanged) surface, whereas magnesium presents an active variable surface during immersion. Thus, this leads to a more complex situation for protein adsorption on the magnesium surface under flow conditions, since fluid flow promotes not only the diffusion of ions/proteins in the medium from the medium to the degradation interface but also the diffusion of released ions (magnesium ions) from the degradation interface to the medium. Furthermore, their influence on the properties of the degradation layer, such as compactness and integrity, also plays a vital role in the degradation of magnesium (Figures 7 and 8). Therefore, protein adsorption behavior and protein performance in the medium under dynamic conditions needed to be studied further to obtain clearer understanding of the comprehensive influence of proteins and flow on magnesium degradation.

It is obvious that the inhibition efficiency of proteins on magnesium degradation (Figure 3), regardless of using BSA or

FBS, decreased from static and semi-static conditions to dynamic conditions, suggesting the deterioration of medium turbulence to the inhibition effect of proteins on magnesium degradation. Furthermore, in comparison with DMEM + BSA, a little change in the degradation rate (0.175–0.195 mm/year) and degradation surface (Figures 2 and 7) was observed for DMEM + 10% FBS irrespective of the immersion condition used, demonstrating that the addition of FBS can weaken the difference in magnesium degradation caused by medium-flow conditions and present more comparable results for different conditions. These different effects for BSA and FBS are attributed to the complex composition of FBS, which contains transferrin, various growth factors and so on, compared with BSA. These results indicate a different effect of protein on magnesium degradation dependent on the implantation site – for example, a slow medium flow in cortical bone or muscle tissue suggests the inhibitive effect of BSA on magnesium degradation, while a relatively fast medium flow in blood vessels implies the accelerated influence of BSA on magnesium degradation. However, the interactions between proteins should be further taken into consideration when the effect of proteins on the degradation of magnesium under in vivo conditions is discussed.

5. Conclusion

In the present study, the comprehensive effects of proteins and flow conditions on the degradation of pure magnesium were investigated by using three different flow conditions, static, semi-static and dynamic conditions. The results revealed the inhibitive effect of protein to magnesium degradation under static conditions (decreased from 1.358 ± 0.068 to 0.444 ± 0.052 mm/year for BSA and 0.188 ± 0.001 mm/year for FBS) and the similar inhibition efficiency of FBS under semi-static and dynamic conditions, while BSA accelerated the degradation of magnesium from 0.301 ± 0.017 and 0.222 ± 0.028 to 0.309 ± 0.074 and 0.849 ± 0.035 mm/year under semi-static and dynamic conditions, respectively. This indicates that the decreased inhibition efficiency of proteins on magnesium degradation is in the order static > semi-static > dynamic (decreased from 35.7 to –2.6 and –281.8% for BSA and from 87.4 to 35.7 and 19.9% for FBS), demonstrating that the medium turbulence decreases the inhibition effect of protein on magnesium degradation. These results imply the flow-dependent influence of proteins on the degradation of magnesium-based implants considering the different flow conditions presented at different implantation sites – for example, fast flow in blood vessels but slow flow in cortical bone. To reduce the difference caused by flow during in vitro testing, a more physiological solution, DMEM + FBS, is recommended as the immersion medium for magnesium degradation due to the more comparable degradation surface (uniform degradation layer and element distribution in the degradation layer) and degradation rate (around 1.80 mm/year) under different conditions. Additionally, the bioreactor-based in vitro method facilitates the understanding of the comprehensive influence of proteins and flow on magnesium degradation under more physiological conditions, although more details about protein performance under flow need to be investigated.

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