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Preliminary Validation of a Dynamic Electrochemical Biodegradation Test Bench in Pseudo-Physiological Conditions

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Abstract

There is a growing interest in the development of next generation stent materials. In vitro tests that accurately predict in vivo conditions, are needed for a full evaluation of a material's corrosion in vivo. In this manuscript a novel approach for the design of a dynamic electrochemical test bench is evaluated in hopes to later characterize and model biodegradable metallic stent materials. This dynamic test bench design allows for real-time corrosion testing with easy variation of temperature, shear stress, and simulated body fluids (SBF), with minimal complications of test sample fabrication. Preliminary tests have shown Tafel generation stable. Further testing of the stability of the test bench were conducted with the incorporation SBF, shear stress, and temperature. Shear stress was applied through variation in fluid velocities at 0 m/s, 0.127 m/s, 0.245 m/s, 0.372 m/s, 0.489 m/s at 37°C. Incorporation of the different SBFs showed no significant difference in corrosion readings; however, variances were observed higher in DMEM and PBS, than in Hanks, respectively. This dynamic test bench showed to be relatively stable under temperature and SBF modification; however, further optimization is needed to decrease variances seen throughout fluid velocity analysis.

Keywords

biodegradable metals; biomaterials; corrosion; in vitro

1. Introduction

Cardiovascular stents are used to treat coronary artery disease, a leading cause of death in United States [1, 2]. This disease is often caused from atherosclerosis, a build-up of plaque that inhibits normal blood flow to the heart. To improve blood circulation, stents are permanently inserted by a catheter and later expanded to widen the atrial lumen. To perform optimally, a stent must have appropriate flexibility for placement and mechanical strength to act as a support structure [3, 4]. Current stents on the market include permanent, drug eluting, and biodegradable polymeric stents [2, 5]. Despite the diversity, all have had various downfalls. Permanent bare metal stents have shown increase risks for re-stenosis, thrombotic occlusion, antiplatelet therapy, and mortality [5, 6]. Although drug eluting stents have been shown to improve these risks, there is still a major concern for drug eluting reactions, such as hypersensitivity and increase of late stent thrombosis [5, 7, 8]. Biodegradable polymeric

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stents have shown limited mechanical properties, in which recoil post implantation can occur [9].

Next generation stent materials are being continuously explored to overcome these limitations. Recent research has shown that after 6–12 months from implantation, a stent is no longer needed to maintain vessel structure [10]. Given, with future advancement, it is projected that biodegradable implants may replace current stent technologies. Biodegradable metals have many advantages including, a high mechanical strength and a decrease in risks for creating a foreign body response. These materials could lessen the need for dual anticoagulants, increase normal vasomotion, and decrease interference during any additional bypass grafts [11].

In order to implement such biodegradable metals for next generation stents, much research is still needed. To fully evaluate the host reaction, a stent would have to be placed within an artery (*in vivo*) and examined. However, often this can increase time, expenses, and uncontrollable variables. Materials are often tested in a simulated physiological environment (*in vitro*). The materials that possess optimal characteristics are then tested *in vivo* for further evaluation. Designing an *in vitro* test is often difficult with materials often showing different results when tested under *in vitro* and *in vivo* conditions [12, 13, 14]. Changes in proteins, osmolality and shear stress in corrosion testing, has shown to be significant [15, 16, 17, 18, 19, 20]. Thus, there is a need to appropriately design an *in vitro* test method to provide a more accurate representation of an *in vivo* properties.

Current testing methods to determine biodegradation behavior of potential stent materials do not accurately represent *in vivo* conditions. Commonly, corrosion tests are performed under static conditions using electrochemical methods in a standard corrosion cell [17, 21]. Although these tests provide corrosion rates that predict long term degradation behavior; they do not adequately test the material under atrial flow conditions. Shear stress exerted by the dynamic motion of fluid can affect the corrosion displayed by the sample, and therefore possibly alter the corrosion behavior under working conditions [16, 17]. Previous work in our laboratory, demonstrated that magnesium (a potential biodegradable material for stent application) surfaces showed different corrosion behavior under flow conditions than in static conditions [22].

In this manuscript a novel approach for a design of a dynamic electrochemical test bench is evaluated which could be used to characterize and model biodegradable metallic stent materials. The experiment design allows real-time corrosion testing of a sample with reduced complications from sample fabrication and implementation into the overall design. Additionally, this experimental design allows for ease when comparing of the effects of shear stress, temperature, and different SBF. Within this experiment, the stability of this design is investigated in hopes of later determining corrosion rates of potential stent materials in atrial flow conditions.

2. Materials and methods

2.1 Test sample

316L stainless steel (316L SS) was used throughout testing due to its predictable inert corrosion behavior [23]. This helped reduce uncertainties created by the corrosion of the sample, allowing for better analyzation of the accuracy of the dynamic test bench. The sample was covered with Teflon mask to expose an even area of 1cm^2 . Previous testing has shown this method favorable, with no presence of uneven or crevice corrosion at the boundary [22]. Previous potentiodynamic experiments have shown 316L SS corrosion rates typically ranging around 0.049 mm/year within SBF at 37°C [24]. Therefore this can be used to further support the values obtained from the control within the experiment.

2.2 Dynamic test bench setup

The overall dynamic test bench assembly consisted of a fluid reservoir filled with 500 mL of the selected fluid, the test chamber, a Gamry Reference 600 potentiostat, a Heidolph 5006 peristaltic pump, and 2.5 wt. tygon tubing. The overall process was as follows: the fluid was pumped from the reservoir, through the test chamber and over the sample, and back to the reservoir again (Figure 1A). The dynamic test bench consisted of three electrodes: a working electrode and graphite electrode (counter electrode) placed across each other on the test chamber, and a reference electrode (saturated calomel electrode) that was placed in the fluid reservoir (Figure 1B). The test chamber was printed from ABS plastic, a material that would not interfere with electrochemical testing of the sample. The dimensions of the rectangular channel that opened up over the exposed sample was as follows: length = 29.5mm , width = 14.2 mm , height = 7.2 mm (Figure 2). Further detail over the design and rationale of the dynamic test chamber design has been reported elsewhere [22].

2.3 Preliminary testing conditions

Tests were conducted in Phosphate Buffer Saline (PBS) at room temperature. These were comparable conditions, previously tested [22]. The standard static corrosion cell (Gamry Apparatus) was used for the control. For preliminary validation, the static tests were conducted in a standard corrosion cell and in the developed test chamber under static conditions. For the static tests in the developed chamber, the pump was turned on to fill the chamber with PBS solution, and then stopped before conducting electrochemical analysis. Tafel plots were generated and corrosion rates subsequently determined and compared between the developed test bench and the standard corrosion cell. Shear stress was incorporated by change in fluid velocities. The dynamic test chamber was evaluated at different constant fluid velocities by maintaining the various settings of the Heidolph pump at: 0 RPM, 100 RPM, 200 RPM, 300 RPM, and 400 RPM, to achieve the respected fluid velocities of 0 m/s , 0.127 m/s , 0.245 m/s , 0.372 m/s , and 0.489 m/s . Each setting was repeated 5 times and the corrosion was represented as a mean $\pm$ standard deviation. The standard deviation obtained was used to better interpret the stability design during each measure.

2.4 In-vitro testing conditions

2.4.1 Temperature analysis—To fully evaluate the apparatus within in vitro conditions, the dynamic test bench was tested at 37°C in PBS. These values were compared to the preliminary tests to assist in an accurate assessment of this machine. To maintain a consistent temperature for the control standard cell, the fluid was heated on a hot plate. The fluid was then transferred to the standard corrosion cell and testing commenced immediately after. For heating in the dynamic test apparatus, the fluid reservoir was placed on top of the heating plate and the temperature was actively monitored with a thermometer throughout both static dynamic conditions. For each setting the experiment was repeated 5 times and the corrosion was represented as a mean $\pm$ standard deviation.

2.4.2 Incorporation of simulated body fluids (SBF)—To incorporate similar osmolality and cellular components a material may experience in vivo, the dynamic test bench was evaluated in Phosphate Buffer Saline (PBS), Hanks, and Dulbecco's Modified Eagle's Medium (DMEM) at 37°C. A range of fluid velocities were compared in each SBF. Additionally, at each velocity the SBF's were compared for a more comprehensive analysis. Testing procedure followed similar protocol described above.

2.4.3 Potentiodynamic polarization—A scan voltage ranging from 1 to -1 V and a voltage scan rate of 10mV/s was maintained for the development of the potentiodynamic curves. This scan rate was chosen in evaluation, in hopes to later observe more corrosive materials [25, 26, 27]. Ideally, once the dynamic test bench is optimized the scan rate will be adjusted appropriately to the material. Gamry DC105 DC Corrosion Techniques software was in the extrapolation of Tafel curves, in which the corrosion rate was subsequently determined.

3. Results and discussion

3.1 Preliminary testing conditions

3.1.1 Tafel Generation—For preliminary testing, corrosion rates of 316L SS were obtained at room temperature in PBS. A Tafel curve was generated for the developed dynamic test bench at 0 RPM, thus further supporting the feasibility of this design. An overlay was created to compare the experimental test bench to the control (Gamry corrosion cell). Even with the change in experimental design, the extrapolation of the Tafel curve was still achievable (Figure 3). Provided the test bench's dynamic setup, its Tafel curve can be observed to have a different corrosion potential, corrosion current, Tafel region, and overall polarization curve than the control. This is to be expected, as once the test bench is proven stabilized in different in vitro conditions, a standard for each test material can be implemented to account for the displacement of I_{corr} . This test bench will be advantageous when testing the change of corrosion between parameters when exposed to in vitro conditions, while the implementation of a standard for the calculation of actual corrosion rates will later be utilized. Corrosion rates determined under static condition using the control, were found to be 0.0344 ± 0.0021 mm/yr., while the developed test bench was 0.0621 ± 0.0057 mm/yr. (Figure 4). Overall, the developed dynamic test apparatus was

validated to be relatively stable during static preliminary conditions with minimal variance in corrosion rates.

3.1.2 Fluid velocity analysis—When introducing shear stress through different flow settings, 100 RPM, 200 RPM, 300 RPM, and 400 RPM. The corrosion readings were respectfully, 0.1260 ± 0.0424 mm/yr., 0.1796 ± 0.0145 mm/yr., 0.1603 ± 0.0166 mm/yr., and 0.0929 ± 0.0145 mm/yr. (Figure 5). There was a larger amount of variance at 100 RPM, while the rest were similar within range. Corrosion rates were comparable with 400 RPM having a slight decrease. There was a general increase in corrosion when comparing a dynamic setting, 400 RPM to static settings of both the control and the test bench (Figure 6). The larger variance at 400 RPM is predicted to be a result from an increase in turbulence.

One way to better quantify the formation of turbulence within the test chamber is by calculating the Reynold's number. The Reynold's number is a ratio of between inertial forces to viscous forces. As inertial forces increase the flow becomes more turbulent and less laminar. The following equation was used to calculate the Reynolds number for changes in fluid velocities, v .

$$Re = \frac{\rho v D_h}{\mu} \quad (3)$$

Where ρ , was the PBS fluid density at 1000 kg/m^3 , D_h was the hydraulic diameter calculated at 0.00956 m , and μ was the dynamic viscosity at $6.965 \times 10^{-4} \text{ Ns/m}^2$.

The approximate transitions for a Newtonian fluid flow are as follows: $Re < \sim 2300$ for laminar, $\sim 2300 < Re < \sim 4000$ for transient, and $\sim 4000 < Re$ for turbulent [28, 29]. Using the equation to calculate the predicted Reynolds number (3), laminar flow was hypothesized to occur at 100 RPM, transient flow at 200 RPM, and turbulent flow at 300–400 RPM settings. Overall, turbulent flow could be a factor for an overall increase in variance for the dynamics settings when compared to the static settings. The variances in values observed at 100 RPM were not expected with laminar flow, leading to the conclusion that possible fluctuation of reading could have resulted from the presence of possible disturbances.

Visual observation of the fluid flow showed a reduced amount of deviances when minimal disturbances were observed (minimum presence of bubbles). The reference electrode was placed within fluid reservoir, allowing for minimal disturbances while induced to the same environmental as the material. A bubble free flow would ensure a more stable conducting electrolyte for voltage application and the resulting current detection. Thus it is hypothesize, that the increase in obtained corrosion values and variance may be a result of the creation or growth of bubbles within the tubing. As the pressure within the tubing got closer to the maximum critical pressure of the bubble, the bubble would grow until the test chamber fluid pressure exceeded the maximum pressure of the bubble, thus leading to collapsion and the reduced variance in readings [30]. The presence of a bubble could possibly explain the increase of variance at 100 RPM despite the minimal to none turbulence. A presence or growth of bubble at any dynamic setting could lead to large variances.

The purpose of incorporating fluid flow during electrochemical testing was to better simulate shear stress that a stent material may encounter. In turbulence, a velocity gradient is created next to the chamber wall creating a wall shear stress on the sample. This resistance can be measured using a combination of Churchill's equations [31]. Churchill's coefficient of friction, which is applicable to both laminar and turbulent flow can be calculated by the following:

$$f = 8 \left[\left(\left[\frac{37350}{Re} \right]^{16} + \left[2.457 \ln \left(\left(\frac{7}{Re} \right)^{0.9} + 0.27 \left(\frac{\epsilon}{D_h} \right) \right) \right]^{16} \right)^{-1.5} + \left(\frac{8}{Re} \right)^{12} \right]^{-12} \quad (4)$$

Where f is the Churchill's coefficient of friction, ϵ is the surface roughness measured at 0.3 μm . Thus shear wall stress τ_w can be calculated by the following:

$$\tau_w = f \rho v^2 \quad (5)$$

The wall shear stress was calculated for each of the dynamic settings (4, 5) (Table 1). Between the dynamic settings of 200RPM-400 RPM the shear stress experienced by the sample was within range of the average shear stress experienced within a vessel. Shear stress within an artery can change throughout the body depending on the atrial lumen diameter, frequently ranging between 0.1–0.7 Pa [32, 33]. Depending where the stent material is placed in the body, this apparatus could properly test the material at the required shear stress through changes in dynamic settings.

3.1.3 Temperature evaluation—Corrosion rates were higher for the control (0.0594 ± 0.0172 mm/yr.) and lower for the test bench (0.0254 ± 0.0041 mm/yr.) when testing was conducted at 37°C PBS, in static conditions. Overall, testing at 37°C showed to have a higher corrosion rate for the control, than when it was tested at room temperature (0.0344 ± 0.0021 mm/yr.). The dynamic test bench portrayed a larger corrosion rate (0.0621 ± 0.0057 mm/yr.) than the one observed at room temperature (Figure 7). The largest variance seen was for the control at 37°C. Hence, the temperature seem to contribute more variance to the control than the test bench at 37°C. It is hypothesized that the larger variances for the control resulted from the limitation of design. The control was unable to receive a consistent temperature source, whereas the dynamic test bench was design for better temperature control and moderation. When comparing the temperatures throughout varying fluid velocities, it was more comparable at 100 RPM and less when parameters approach 400 RPM. An increase in variances for 37°C is seen from 100 RPM-300 RPM. This variance is seen to decrease greatly at 400 RPM (Figure 8). An overall increase in corrosion rate was seen at 37°C for both 0 RPM and 400 RPM for the test bench (Figure 9).

The corrosion values obtained at room temperature and 37°C were expected to be similar, due to 316L SS high temperature resistance. Although significant temperature changes have shown to affect corrosion values for 316L SS in a multitude of studies [34, 35, 36], the difference between 37°C and room temperature is not significant to affect 316L SS in short

tests. Tests conducted at 0 RPM-300 RPM in both the dynamic and control bench were comparable, as expected. However, 400 RPM for the test bench was higher than anticipated, and hypothesized to be contributed from possible disturbances discussed previously. Temperature changes can be significant in corrosion values when testing a potential stent material in vitro. Magnesium alloys are much more temperature sensitive than 316L SS, showing higher corrosive values at lower temperatures when tested in vitro. This is even more prevalent when magnesium contains impurities such as nickel or iron [37].

Temperature is even more influential on values when considering the overall effect it can have on the fluid dynamics of the system. Slight increases in temperature can have a large effect on dynamic viscosity of future potential fluids, which in turn can affect the shear stress on the sample. PBS dynamic viscosity only changes slightly by ~ 0.0002 Pa-s, thus this parameter was considered to have little effect, allowing for an easier depiction of variances in corrosion rates caused from the design rather than the effect of temperature itself. If a fluid, such as blood was tested, corrosion rates could be more temperature sensitive. Blood's dynamic viscosity can change significantly with temperature variation [38]. The inclusion of temperature is critical to accurately simulated applied shear stress and fluid dynamics to get representative corrosion values in vitro.

3.2 SBF evaluation

3.2.1 Control compared to test bench—Testing was continued at 37°C with further analysis of the SBFs. Corrosion rates in the control were all slightly higher than in the test bench, throughout all SBF. All corrosion rates in the control were within range and all the corrosion rates within the test bench, were comparable. Variance increased in both the control and test bench, from PBS to Hanks and then Eagles Medium with the highest (Figure 10).

3.2.2 Comparison of fluid velocities at each SBF—When comparing fluid velocity settings with each SBF, a general increase in corrosion rates was observed for PBS and DMEM. Hanks solution showed no observable trend, 100 RPM-300 RPM was all within range, with 400 RPM having the lowest corrosion value 0.0856 ± 0.0370 mm/yr. DMEM showed increased variances with increasing fluid velocities (Figure 11). Overall there was no trend with change in fluid velocities, all values were comparable up to 400 RPM. DMEM showed the largest variance in corrosion at 400 RPM. 0.61908 ± 0.265886 mm/yr. throughout each SBF there was an increase in corrosion rates from a static to a dynamic setting. A previous study evaluated the corrosive effects of SBFs on 316L SS, found that Hanks showed higher corrosion resistances than PBS, due to its additives of sulfate ions and glucose. It was predicted that KH_2PO_4 and Na_2HPO_4 in both Hanks and PBS increased corrosion resistance through film development on the material's surface [39]. Experimental results supported this claim with Hanks being most corrosive resistant, showing lowest variances (on average) followed by PBS and DMEM, showing highest variances (Figure 12).

3.2.3 Comparison of SBF at each fluid velocity—Analyzation of all SBF at each fluid velocities showed to be rather comparable at the control, and 0 RPM-300 RMP testing parameters. This is not unexpected because all SBF shared similar dynamic viscosities and

densities, thus exhibiting a similar shear wall stress. The range of readings at 400 RPM is predicted to be a result of turbulence seen throughout testing. There was an observable increase in corrosion rates as fluid velocities increased from static-300 RPM (Figure 13).

3.3 Design rational and limitations

The test chamber design allowed for a decrease in complications in test sample fabrication. The flat test chamber and electrode setup, allowed for any flat sheet of metal to be implemented into the design. The overall test bench assembly allowed for real-time testing, while shear stress and temperature factors were applied. Due to the design, the reference electrode had to be placed in the fluid reservoir and not directly in between the two electrodes (as seen with the control). An increase distance between the working electrode and counter electrode can increase IR drop. Since similar IR drop is consistently present throughout all dynamic testing and does not inhibit the ability for Tafel generation, the effect of IR drop is assumed to be consistent in all tests conducted and hence it is assumed that this factor does not contribute to the variances experienced within the experiments. Once the test bench was optimized, this IR drop can be quantified and representative corrosion values could be determined. Overall, this electrode placement can be seen favorable, because it reduced the interference the reference electrode may experience if placed inside the dynamic test chamber.

Dynamic settings showed possible presence of turbulence and disturbances. However, Tafel curve generation was possible throughout all settings. Thus, quantifying corrosion rates is feasible, however optimization of the test chamber may be needed to reduce variance. Although turbulence was predicted in the following experiment, it is hypothesized that this may not be as significant when performing tests with a similar Newtonian fluid with equivalent dynamic viscosity and fluid density of blood. At 37 ° C blood has a dynamic viscosity 2.78mPa·s and a fluid density of 1060kg/M³, both properties higher than the previous tested fluid [40]. By continuing previous calculations, the Reynold's numbers for the fluid would be much lower within the laminar flow range (Table 2). This decrease in turbulence flow could possibly decrease variances. Despite the changes in dynamic viscosity and fluid density the desired shear wall stress could still be applied to the sample.

Despite the design's potential ability to test future stent materials in vitro, optimization must be made to minimize possible variances observed at current conditions. It is predicted that the reduction of the outer layer cut outs on the test chamber may reduce possible disturbances. This would allow a greater pressure to be applied to insure an airtight seal between the sample and the test chamber. Additionally, optimizations should be made to insure temperature stability throughout the use of the design. Although the experimental test apparatus showed less variance than the control at 37C, the implementation of a thermostable water bath may decrease variances seen between readings. Lastly, further investigation is needed to analyze the effects of a fluid with the same dynamic viscosity and fluid properties in vivo to further evaluate this apparatus in possible laminar flow conditions.

4. Conclusion

The test bench was evaluated within in vitro conditions through changes in shear stress, temperature, and SBF. Preliminary tests showed the developed test chamber to be relatively stable and Tafel generation feasible. In vitro conditions were then conducted and a further evaluation was made over the stability of the dynamic test bench through changes in shear stress, temperature, and SBF. Variances in the dynamic test bench were higher with the incorporation of temperature and shear stress, with no notable difference between SBF. Overall, this dynamic test bench proved favorable in offering real-time corrosion testing with the incorporation of shear stress and temperature control with ease of sample fabrication. However, further considerations are needed to fully assess what deviances are attributed to the design flow conditions compared to the actual setup.

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Figure 1.

A.) Overall test bench assembly, B.) Components of experimental test chamber

Figure 2.

A.) Previously developed 3D CAD design of test chamber [22] B.) Design allows for even exposure of shear stress on 316L SS sample

Figure 3.
Comparative Tafel overlay for both the control and experimental test bench in static conditions

Figure 4.
Comparison of average corrosion rate readings for control and test bench in static conditions

Figure 5.
Comparison of corrosion rate readings at varying fluid velocity settings

Figure 6.
Depiction of corrosion readings difference from static to a dynamic setting

Figure 7.
Comparison of corrosion rates when tested at room temperature and 37°C, in static conditions

Figure 8.
Comparison of room temperature and 37°C corrosion readings at varying fluid velocity settings

Figure 9.
Corrosion reading differences at room temperature and 37°C for static and dynamic settings

Figure 10.
Corrosion reading for the control and test bench tested in PBS, Hanks, Eagle's Medium

Figure 11.
Corrosion reading of different fluid velocity settings for each SBF

Figure 12.

Depiction of corrosion reading corrosion reading differences between each SBF for changes in a static to a dynamic setting

Figure 13.

Comparison of each SBF corrosion readings at each dynamic fluid velocity setting

Table 1.Calculated Re and τ_w for SSL 316 in PBS

Setting	v (m/s)	Re	τ_w (Pa)
0 RPM	0.000	0.000	0.000
100 RPM	0.127	1744.344	0.074
200 RPM	0.245	3354.507	0.320
300 RPM	0.372	5098.851	0.652
400 RPM	0.489	6709.015	1.040

Table 2.Projected Re and τ_w “Similar” Blood Newtonian Fluid

Setting	v (m/s)	Re	τ_w (Pa)
0 RPM	0.000	0.000	0.000
100 RPM	0.127	463.249	0.296
200 RPM	0.245	890.863	0.570
300 RPM	0.372	1354.112	0.866
400 RPM	0.489	1781.726	1.139