Tensile adhesion of type I collagen to titanium alloy and calcium phosphate coated surfaces with different roughness values

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Abstract. The purpose of total joint arthroplasty is to reduce pain and restore function. Its success depends on the formation of a new bone that stabilizes the prosthesis. The proposed solution for this important problem is to have bio-coated implant surfaces which are more conductive to bone growth. Additionally, collagen has long been used as a matrix for medical applications, because of its biocompatibility and adaptability. In this study, a test method for measuring the tensile adhesion strength of collagen to titanium alloy and calcium phosphate coated surfaces with different roughness values was developed, in order to evaluate how well the collagen adheres to the metallic and bio-coated surfaces. A precision motion system was used to stretch gels that were adherent to the plate surfaces. The tests were done in DMEM solution. The adhesive strength between the collagen gel and plate was significantly higher for calcium phosphate coated surfaces. Adhesive strength was highest in the sample with the highest roughness value.

Keywords: Titanium alloy, calcium phosphate, adhesion, tensile strength, surface roughness

1. Introduction

The purpose of total joint arthroplasty is to reduce pain and restore function. Its success depends on the formation of a new bone that stabilizes the prosthesis. If stabilization does not occur, the prosthesis must be removed. The most likely cause of implantation failure is implant loosening [15]. Loosening may be caused by a number of factors such as severe bone loss or atrophy, bone fracture, infection, disassembly of a modular component, and implant fractures [1]. One of the most common causes of loosening is poor implant fixation. In the case of a joint prosthesis attached with bone cement, poor fixation is frequently due to improper cementing technique at the time of surgery [6]. For uncemented prosthesis, stability is achieved by either press-fit or biologic fixation. Poor fixation for uncemented prosthesis is due to bone resorption at the interface of either the roughened or porous surface of the metallic implant [2,7].

Close contact between a porous surface and bone is required for assurance of bone ingrowth. For hip acetabular components, gaps as small as 0.5 mm between the porous implant and bone surface may not fill with bone [17]. Histologic studies of retrieved uncemented hip femoral implants had shown that bone ingrowth actually occurs on only a limited portion of porous coated surfaces. Poor bone ingrowth is attributed to poor opposition of the porous surface coatings, which are more conductive to bone growth.

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Calcium phosphate's osteoconductive behavior was first reported by Albee and Morrison [3]. Some studies have been investigated the mechanical behavior of the calcium phosphate coating and enhancement of bone implant interface [4,5,9,11,12,14,16].

Type I collagen is the major structural protein in bone. Due to its biologic, chemical and physical properties, it has been used widely in the repair and regeneration of bone. Some studies showed that collagen, also, promotes cell proliferation [10,18].

Collagen was shown to promote osteogenesis after surgery in a dog model [8]. It was concluded that collagen could be used as a glue, or matrix without interfering with healing.

Transforming Growth Factors (TGFs) are also being investigated for their effects on increasing joint prosthesis stability. TGFs are polypeptides that induce some cells to undergo growth [13]. TGFs can not be manufactured commercially and applied to the surface of prosthesis. However, TGFs can be mixed with collagen and applied to the surface at the time of surgery. Collagen is used to glue TGFs to the prosthesis, so that TGFs remain at the bone and prosthesis interface long enough to promote bone growth and implant stability.

A number of questions remain unanswered about how well the collagen adheres to the metallic surface. In this study, tensile adhesion strengths of a collagen type I to titanium alloy and calcium phosphate coated surfaces with different surface roughness values are measured and compared with each other in order to determine optimum parameters.

2. Materials and methods

Ti–6Al–4V was selected for being one of the two most frequently used metals in joint prosthesis along with Co–Cr–Mo. Commercially manufactured calcium phosphate ceramics are combination of hydroxyapatite (HA) and tricalcium phosphate (TCP) and have been studied as osteoconductive surface coatings. Average roughness value of rectangular shaped (20 mm × 10 mm) titanium alloy plates are 2–3 Ra and 8–10 Ra. Calcium phosphate was applied with a plasma-spray process that produce a thin hard coating on the titanium alloy surface. The coating thickness was approximately 50 μm.

The holding chambers were formed from plastic laboratory culture chambers. Liquid Slygard^{TM} Silicon Elastomer mixed with a curing agent was inserted into the chambers and allowed to dry for 4 days. Once the silicon was dry, a section (22 mm × 10 mm) of the silicon was removed from the chamber to allow fixation of the lower plate to the bottom of the culture chamber. The removed portion of the silicon thus formed a close fitting wall around the plates, enabling the formation of collagen gel on the plate surface.

The collagen used for all experiments was Vitrogen^{TM} 100 by Celtrex Laboratories. Vitrogen^{TM} 100 Collagen is a sterile solution of purified, pepsin-solubilized bovine dermal collagen dissolved in 0.012 N HCl. The collagen gels were prepared with dilute Vitrogen^{TM} 100 collagen. 1 ml Vitrogen^{TM} 100 collagen was mixed with 0.7 ml of DMEM solution to give 1.76 mg/ml ratio for collagen concentration. The collagen gel solution was injected between the titanium plates, which had 1.0 mm clearance. The gel was formed in the incubator under CO_2 free, 37°C, and 100% humidity conditions. The time for collagen gel formation was 3 hours.

The experimental setup for this experiment is shown in Fig. 1. Holding chamber contents a bridge and a base. For vertical pulling of the titanium–collagen gel couple, the rod was threaded into the top piece of titanium. A nut was placed on the threaded rod above the bridge. An aluminum spacer was machined in order to have the same clearance between the plates. A linear actuator at 0.5 mm/s constant vertical
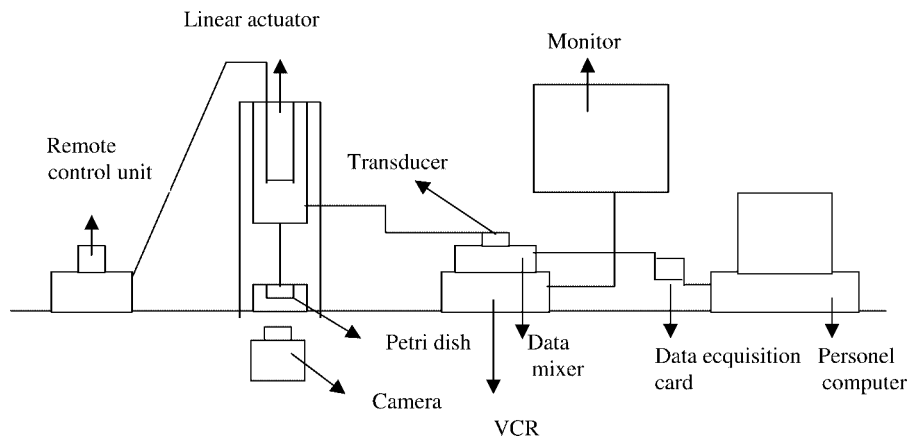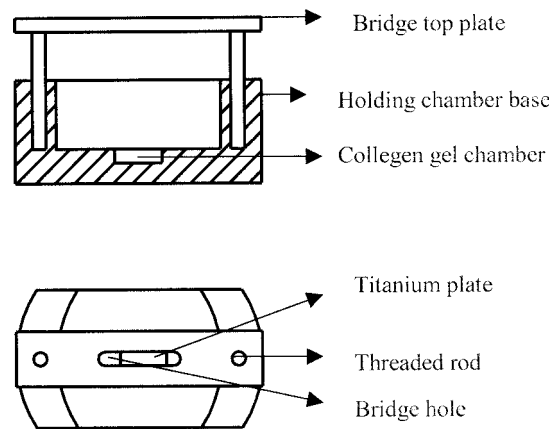
Fig. 1. Experimental set up.

Fig. 2. The holding chamber and the bridge.

speed was used to apply tension on the titanium alloy or bio-coated plates and collagen gel couple. A force transducer was attached to the linear actuator to measure the tensile force in the string. The transducer was attached to a personal computer. The real-time force on the string was recorded by using a data acquisition card. The data were saved on to a file in ASCII format. These data were later converted into dyne through a program written in MATLAB. Also, a video camera and a data mixer wired to the VCR were used to monitor and calculate the stretched distance. A simulation was run without any gel in order to get data on the effect of gravity during the experiment. These data were subtracted from the results.

The bottom plate was placed in the holding chamber. The holding chamber was placed in the base. The rod suspended the top plate. The aluminum spacer was placed between the plates. The nut was used to adjust the plates so that it just touched the spacer. A string was attached to the nut. Figure 2 shows the holding chamber and the bridge. The collagen mixture was poured into the chamber. The bridge and holder chamber was placed into the incubator. After incubation, it was placed and oriented on the vertical platform. The loop end of the string was then placed around the force transducer. 20 trials were made for each type of plate.

The strain (ε) was determined by using the following equation:

$$\varepsilon = (L - L_0)/L_0, \tag{1}$$

where L_0 and L denote, respectively, the length of the gel before and during stretching.

3. Results and discussion

Figures 3 and 4 compare stress-strain relationships for the titanium alloy and coated surfaces for different roughness values in DMEM solution. Maximum of average stress values are 2.48 dyn/mm^2 and
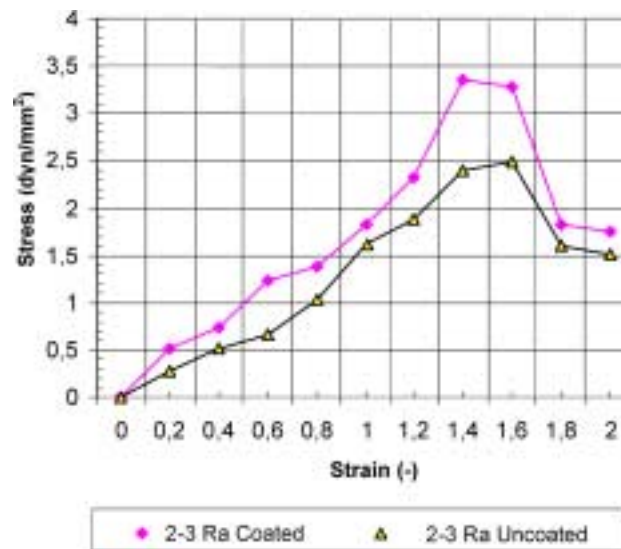

Fig. 3. Strain versus stress for coated and uncoated surfaces having roughness of 2–3 Ra.
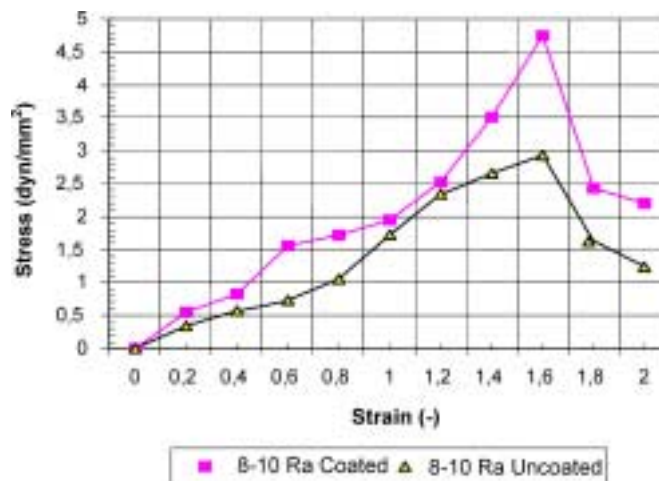

Fig. 4. Strain versus stress for coated and uncoated surfaces having roughness of 8–10 Ra.

2.93 dyn/mm^2 for 2–3 Ra and 8–10 Ra titanium alloy, respectively. However, for calcium phosphate coated surfaces maximum stress values increase up to 3.36 dyn/mm^2 and 4.74 dyn/mm^2 according to the surface roughness values of 2–3 Ra and 8–10 Ra, respectively. Figures 3 and 4 show the comparison between titanium alloy and calcium phosphate coated plates with the roughness of 2–3 Ra and 8–10 Ra, respectively. Also, Figures 5 and 6 compare the roughness values of 2–3 Ra and 8–10 Ra for titanium alloy and calcium phosphate coated plates, respectively.

4. Conclusion

An experimental setup to perform an adhesion strength test between a collagen gel and a plate was constructed. The entire set up was completely automated and computer controlled. The adhesion increases
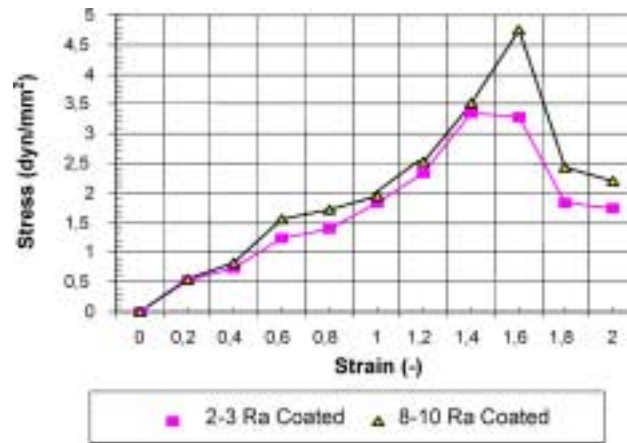

Fig. 5. Strain versus stress for coated surfaces having roughness of 2–3 Ra and 8–10 Ra.
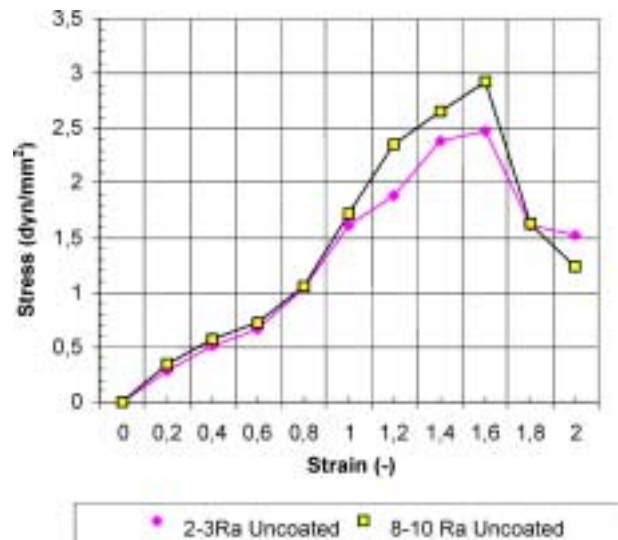

Fig. 6. Strain versus stress for uncoated surfaces having roughness of 2–3 Ra and 8–10 Ra.

for an increasing roughness for calcium phosphate coated and titanium alloy surfaces. The adhesive strength between the collagen gel and plate was significantly higher for calcium phosphate coated surfaces. Adhesive strength was highest in the sample, which had the highest roughness value. Strain value against yield point was increased in favor of calcium phosphate surfaces. This indicates that bio-coated surfaces are more conductive to bone growth. In other words, this shows the importance of mechanical adhesion in order to improve the behavior of metallic implants. This study pointed out analogous behavior between bio-coated and uncoated titanium alloy surfaces. Further experiments can simulate the *in vivo* environment by replacing the upper plate with bone tissue.

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