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RESEARCH ARTICLE

POLYMER USES IN TISSUE ENGINEERING.

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Abstract

The biodegradable polymer, poly(caprolactone) (PCL), was used to dip-coat silicon-stabilized resorbable calcium phosphate ceramic pellets and scaffolds implants. The PCL coatings increased in thickness on the dense ceramic pellets with the number of dipping and did not detach from the ceramic substrates during mechanical testing. The distribution of PCL in the porous scaffolds was not homogeneous, i.e. mostly located along the outer surface areas and at the interconnection of struts. The scaffolds with thick PCL coatings were handled in ways analogous to those that are used in clinical applications, such as drilling, screwing, cutting and grinding, etc.; it was found that this could be done without powdering or shattering. The compressive failure features of the thickly PCL-coated scaffolds were quite different from those of uncoated scaffolds and those with thin PCL coatings. For uncoated scaffolds or those with a thin PCL coating, the compressive strength decreased quickly from the yield point to zero after failure, but for the thick PCL-coated scaffolds, it became nearly constant strength after a quick decrease from the yield point, and started increasing towards the end of a constant strain region.

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Introduction:-

It has been reported that an ideal synthetic bone-graft material would support the activity of osteoblasts in the development of new bone, while simultaneously being resorbed by osteoclasts as part of the lifelong orderly process of bone remodeling [1]. Such a material has been developed and is being produced [2]. These silicon-stabilized, multiphase, calcium phosphate-based biomaterials are completely resorbable by osteoclasts both in vitro and in vivo and promotes the uniform deposition of newly mineralized bone matrix, thus enabling rapid integration of the implant with the surrounding host boney tissue in vivo [1,4&6]. The implant material has two characteristic features: a phase composition which is a mixture of calcium hydroxyapatite and silicon-stabilized tricalcium phosphate, and a micro-porous morphology derived from fine interconnected particles [1].

Based on the concept of biomimicry, efficient bone-graft scaffolds will have the architecture features of natural bone [6-8]. It is well known that natural bone is itself a composite of an inorganic matrix, which is mainly poorly crystallized, non-stoichiometric carbonate apatite and forms a framework of hard tissue, and collagen, which is a natural polymer and fills the pores of the framework [9&10]. The inorganic matrix of cortical bone consists of a porous structure with an interconnected porosity of about 65% and pore sizes of 200 – 250 μm while pore sizes in

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trabecular bone are larger, in the range of 500 – 600 μm [10&11]. While both porosity and pore sizes can vary substantially with bone type. It is known that those pore structures best suited to bone tissue ingrowth must contain highly interconnected porosity with minimum pore interconnection sizes of approximately 100 μm [12-14].

While several processing routes for the production of porous ceramics exist [15–18], the polymeric sponge replication technique, first developed by Schwartzwalder and Somers in 1963 [19], produces a 'reticulated' ceramic consisting of interconnected voids surrounded by a web of the ceramic material. Porous ceramics fabricated using this technique possess an architecture very similar to that of natural bone and have the advantage of being able to adjust the porosity and pore size of the final sintered product to best suit the intended application [20].

Unfortunately, porous ceramics produced by any technique have limited mechanical properties because of the presence of the pores and the inherent brittleness of the ceramic web. As such, such porous bone graft substitutes are difficult to handle, are limited to non-load bearing applications, and cannot tolerate the deformation resulting from standard orthopedic fixation techniques [21].

In an effort to overcome these shortcomings, biodegradable polymeric coatings were applied to the surfaces of porous implants [22]. These coatings are not intended to improve the mechanical properties of the scaffolds necessarily, but to provide a safety glass effect that will minimize the fragmentation and release of particulate debris from the porous ceramic during deformation while maintaining the interconnected porous structure of the bone graft. In addition to preventing the release and subsequent migration of particulate debris in vivo, the incorporation of a "safety glass" coating may improve the ability of the scaffold to be shaped by the surgeon using standard techniques. In addition, the polymer coating may be used for the controlled delivery of site-specific growth factors to accelerate the rate of healing [23-30].

Tencer et al. have reported the use of two other polymers, polymethylmethacrylate (PMMA) and polylactic acid (PLA), to improve the toughness of porous hydroxyapatite implants by either filling the internal pores, or producing a shell around the porous piece [21]. Both of these polymer-impregnated materials exhibited an increase in the implants compressive strength compared to the uncoated ones. However, PMMA is non-biodegradable and, therefore, does not take part in the bone remodeling process. Other researchers have demonstrated that PLA can enhance the mechanical degradation of PLA-filled carbonate hydroxyapatite composites in both in vitro and in vivo experiments [21]. The rapid degeneration of mechanical properties was a consequence of the rapid degradation of PLA and the extremely acidic environment it produces as it degrades [26].

Unlike PLA, polycaprolactone (PCL) does not generate an acidic environment upon degradation [26] and, therefore, may be advantageous for the sustained delivery of bone-growth-promoting proteins and polypeptides. In addition, PCL is the toughest readily available biodegradable polymer, and is approved by the US Food and Drug Administration (FDA). Because of these reasons, the present study focused on the preparation of PCL-coated dense pellets and porous implant "scaffolds" in order to maintain an interconnected porous structure in the case of a scaffold and minimize the release of particulate debris following deformation.

Materials and Methods:-

Materials

Poly(caprolactone), with an average molecular weight of 50, 000 and an elongation to failure of 800%, was selected for the present study and kindly donated by Solvay Caprolactones, UK. The silicon-stabilized calcium phosphate powder was used to produce both dense pellets (13 mm Dia. x 1 mm) and porous scaffolds 9 mm Dia. x 13 mm). Figure 1 shows the porous structure of the scaffolds prior to coating with PCL.

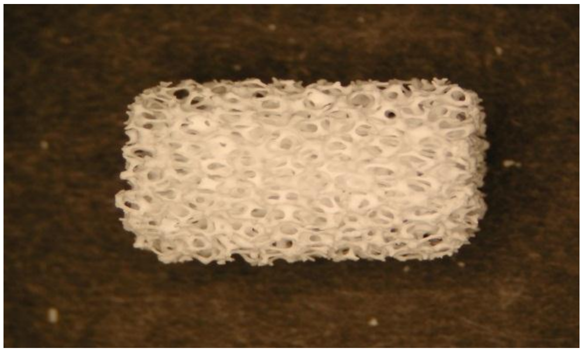


Figure 1:-SEM photograph of porous bone-graft scaffolds without polymer coatings [20]

Coating Technique

In the present study, "dip" and "dip and spin" coatings were applied to the dense pellets and scaffolds, respectively. In each case, the thickness of the final polymer coating was found to be affected by such variables as the initial coating thickness, the withdrawal rate, spin speed and time, PCL solution viscosity, surface tension, and the solvent evaporation rate. In the dip-coating process, the dense pellets were immersed in the PCL solution and slowly withdrawn from the solution and allowed to dry in air. In the spin-coating process, the scaffolds were immersed into a PCL solution contained in a sealed metal vessel, which was evacuated prior to the introduction of the PCL solution. The samples were subsequently, spun after withdrawal from the solution, and allowed to dry in air.

Coating Distribution and Thickness

The distribution of PCL in the scaffold structure was examined with a stereoscope. An optical microscope was used to measure the thickness of the coatings on the dense discs. The coated samples were mounted in cold-setting Epoxy and subsequently ground and polished using the standard metallographic techniques. As the PCL polymer used in these experiments is uncolored and clear, identifying the coverage, the uniformity of the coating and the thickness of the coating is difficult. Because of this, a method of coloring the polymer was attempted.

Of the several dyes available, Waxoline Black (UK) was chosen. The dyed PCL solution was prepared using the following procedure: Waxoline Black was added into methylene chloride and stirred for minutes, and subsequently, PCL powder was dissolved into the dyed methylene chloride with magnetic stirring at room temperature in a fume hood. There was no sediment and aggregation of dyes in the dye solvent for an extended period of time. In addition, the dying of black-colored PCL solution was not influenced by the black dye.

A 5% (w/v) PCL solution was used in the dip-coating process. The dense pellets were used to investigate the influence of processing parameters on the formation of PCL coatings during the dipping process. This was performed to provide some data concerning the interaction between PCL coatings and the ceramic substrates.

Mechanical Testing

For porous scaffolds, a compressive test was performed using an Instron Universal Tester. The scaffolds were glued with Epoxy onto two SEM stubs. Care was taken to ensure that the two faces were parallel and aligned. The samples were allowed to cure overnight. The strength of the dense discs was measured using the compressive strength mode test. The mechanical characteristics of the PCL-coated scaffolds were also evaluated by simulating the ways in which they might be treated in a clinical application, such as shaping, drilling, grinding, etc.

Results:-

Relationship of the Final Coating Thickness to the Number of Dipping

Pellets that had been dipped 1, 3 and 5 times in 5% (w/v) PCL solution were fractured and mounted using Epoxy cement. The cross-sections of the pellets were obtained by means of grinding and polishing the cured blocks. The coating thickness was measured with a graduated optical microscope eyepiece; the data is shown in Table 1. There was no observable coating on the surface of the pellets, as shown Figure 2. It appears that the polymer has been pushed into the center of the pellet during the curing of the Epoxy, leaving almost no polymer in / near the surface. This was in contrast to the fact that the polymer diffused only into the near surface following dipping into the PCL solution. The cold-setting Epoxy did not cause the polymer in the thickly PCL-coated pellets to move to the centre line of the pellets as it did with the pellet with only thin coating (1 coat), Figure 3.

Table 1:-Relationship of PCL coating thickness to dip numbers

Coating Number	1 coat	3 coats	5 coats	3 coats &H-Treated
Thickest among 20 measurements	16	80	110	60
Thinnest among 20 measurements	4	25	68	8
Average thickness (µm)	11	46	89	29

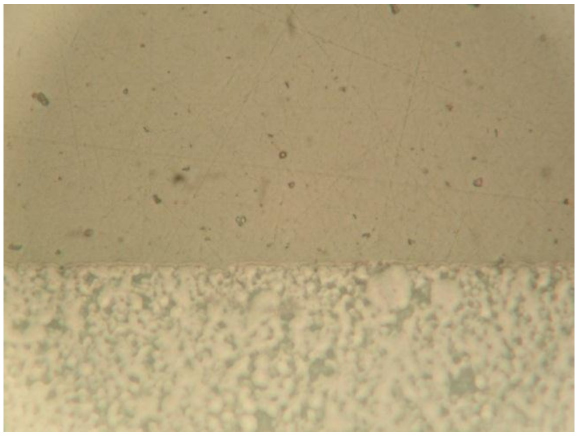
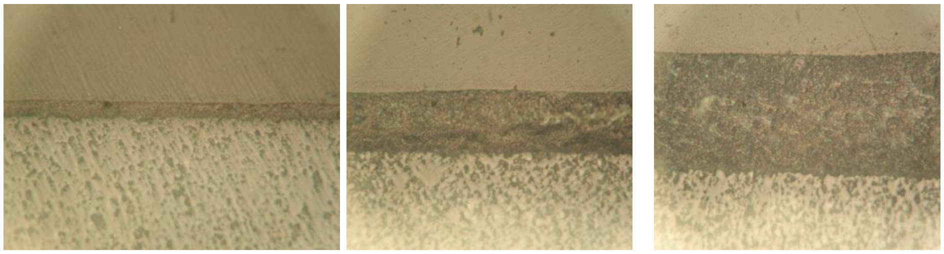


Figure 2:-A cross-section of the coated disc, Mag. 40X

Figure 3 shows the cross sections of PCL coated pellets dipped a number of times; this shows that the thickness increased with the increase of number of times dipped. There were pores through the thickness of the PCL coatings.

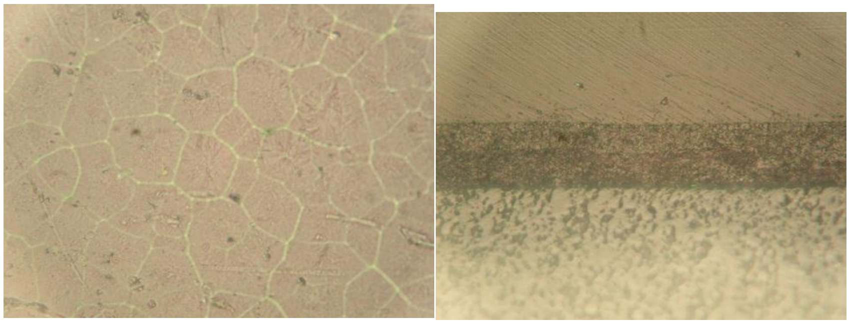


(a) 1 coat (b) 3 coats (c) 5 coats

Figure 3:-Cross sections of the PCL coated discs with different coats, Mag. 40×

Heat-treatment of the Dyed-PCL Coated Plates

The dyed-PCL coated plates (3 coats) were heated to 110°C for 10 minutes and cooled to room temperature in an oven. The black coating exhibited a shining gloss visible with the naked eye but when viewed via a low magnification optical microscope, the coating showed a dense and very flat surface with micro-cracks distributed evenly all over the surface (Figure 4a). Heat-treatment reduced the thickness of PCL coating as compared with that of just-coated sample (Figure 4b and table 1). A cross section showed that the heat-treated PCL coating was denser than an un-heat-treated one as would be expected, since the solvent would have been largely removed by the heat-treatment.

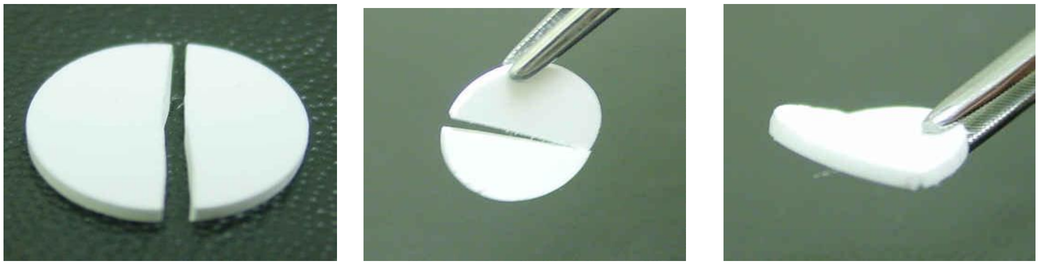


(a) Top surface, Mag. 40X (b) Cross section, Mag. 40X

Figure 4:-Light microscope photos of PCL coated pellets (3 coats) after heat-treatment in air.

Mechanical testing of the coated pellets

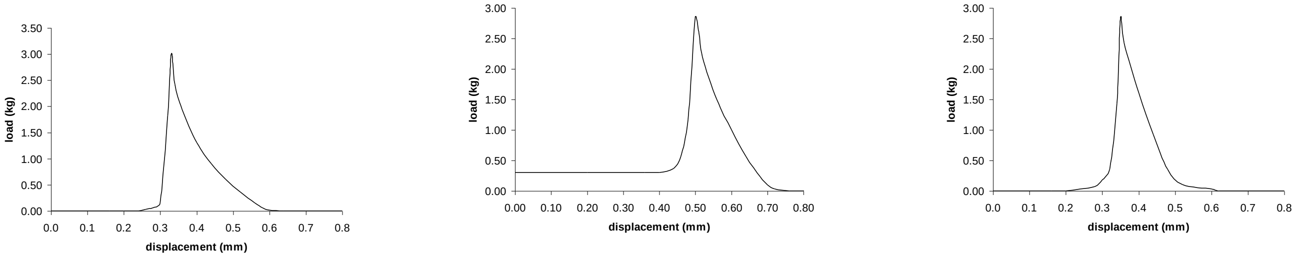
The as-received uncoated and coated pellets were broken into two by using a three-point bending testing. With only one dip coating, the film still managed to hold the pieces together, while the plates coated 5 times were held together quite strongly by the PCL coatings even after fractured, Figure 5c. The PCL coatings did not detach from the substrates, indicating a strong adhesion between the PCL coatings and the ceramic substrates.



(a) un-coated (b) 1 coat (c) 5 coats

Figure 5:-Coated and un-coated pellets after the mechanical test

Figure 6 shows the load-displacement curves of dense pellets with and without PCL coatings after a three-point bending test. It can be seen that the load went immediately from the failure value to zero for the as-received plates and those coated once but to a value of about 0.3 kg for the plates coated 5 times for a period until it finally fell to zero as the PCL coating was finally severed. Dense pellets had much a higher stiffness than the PCL coatings so initially they bore almost the entire load, until fracture at which time they broke leaving only the PCL coatings during the compression test. Therefore, the PCL coatings almost had no influence on the failure loads of dense pellets; these were in the range of 2.20 - 3.13 kg.



(a) un-Coate (b) 1 coat (c) 5 coats

Figure 6:-Load-displacement curves of plates with and without PCL coatings during the compression test.

Dip Coat of Porous Scaffolds with the PCL Solution

The PCL coatings were successfully applied to the surfaces of dense ceramic pellets by the dipping process. The thicker PCL coatings after 5 coats did not detach from the ceramic surface with high molecular weight PCL, whereas with the low molecular weight PCL, some flaking away occurred; this suggests that high molecular weight PCL would be the more suitable coating for porous scaffolds when producing the "safety glass" coatings.

The coating of all internal surfaces of porous scaffolds requires firstly a complete penetration of PCL solution into the porous structure. The penetration of a viscous solution into any such porous structure depends on not only the solution concentration and the nature of the porous structure but also on the coating techniques.

Mechanical properties of PCL coated scaffolds

The mechanical properties of scaffolds dip-coated with PCL were evaluated by compression testing and in ways in which they might be handled / shaped in a clinical application. Scaffolds with thicker PCL coatings were struck with a pointed implement to make a hole through the scaffold, or drilled and then a screw is seated into the hole. This could be achieved without dispersal of any ceramic fragments; all these being held by the polymer coating. In addition, mounted samples could be reduced by grinding to a thickness of 3 mm without disintegration.

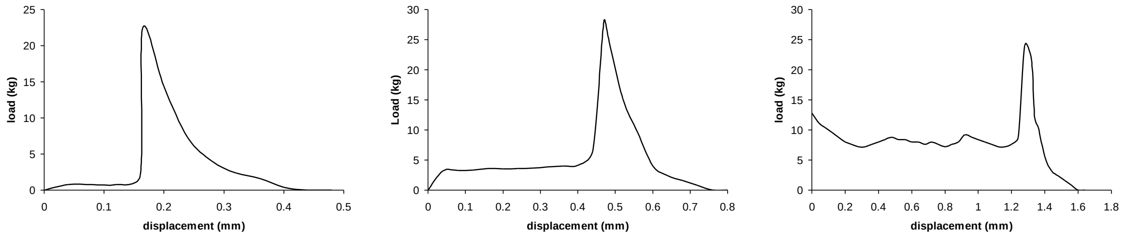
Figure 7:-As-received porous scaffold after the compressive test.

Figure 8:-A porous scaffold with 1 PCL coat after the compressive test.

Figure 9:-A porous scaffold with 5 PCL coats after the compressive test.

Compression test was performed on the PCL-coated porous scaffolds. The as-received scaffolds experienced typical compressive brittle failure, Figure 7, while the scaffolds with one coat broke but maintained their structural outline

Figure 8. The scaffolds coated 5 times as they were compressed as it can be seen in Figure 9. Thus, the thick PCL coatings (5 dipping) were strong enough to fix the broken pieces parts of the original brittle struts tightly in position, without general shattering, while the scaffold with only one coating exhibited a large crack. In contrast, the uncoated scaffold broke completely in two pieces and discharged hydroxyapatite fragments. Again, the ceramic scaffolds struts bore almost all the load before failing during a compression test because of their stiffness was much higher than that of the PCL coating layer. Therefore, it was not surprising that there was no increase of compression strength (1.70 - 2.70 MPa) for porous scaffolds when coated with flexible but less strong PCL coatings. The striking change for load / displacement curve under compression testing occurred in Figure 10C, where it can be seen that there is a considerable, nearly constant, residual resistive force after the yield point, even showing an increasing strength as the compressive displacement increased, i.e. the region on the left-hand side of the curve. In contrast, for uncoated and thinly PCL-coated scaffolds, the load decreased quickly from the yield point to almost zero on the load / displacement curve, Figure 10a and 10b.



(a)

un-coated (b) 1 coat (c) 5 coats

Figure 10:-Load-displacement curves of scaffolds with and without PCL coatings during compressive testing.

Discussion:-

The primary goal of our experiments was to learn if porous ceramic scaffolds coated with a thin biodegradable polymer coating, i.e. could be shaped in ways that were likely to be used by the surgeons during an actual operation would provide increased user-friendliness as well as improved mechanical strength. The fact the PCL-coated implant showed much improvement in its ability to be cut, shaped, drilled and screwed as might be required during use in a practical surgical application. The PCL coatings adhered well to the surface of silicon-stabilized calcium phosphate ceramics even when the coated plates were broken during the mechanical test, Figure 5 (b, c). The PCL coatings would anchor to the surface of porous implant more tightly because the micropores on the strut surfaces provided more contact sites for PCL coatings to attach.

Polymers have been used in the past to impregnate or infiltrate a brittle matrix with a continuous pore system and form a second continuous phase [31-39]. The improvement in reported mechanical properties was outstanding, especially the strength properties, which increased by a factor of nearly 4 times. The polymer used for impregnation was poly(methylmethacrylate) (PMMA) which had a mean flexural strength of 54 MPa and a flexural modulus of 4 GPa. For comparison, earlier work on synthetic hydroxyapatite Goniopora (CHAG), either micro-coated using PMMA or PLA to cover all internal surfaces, or externally coated to produce a shell, with the objective of reducing the brittleness of the material may be considered [31&32]. CHAG internally micro-coated with PMMA showed 3.84 times increase in compressive strength while specimens with external PLA coatings had 1.81 times the compressive strength of uncoated CHAG specimens. PMMA is mechanically stronger than PLA, which is the strongest among the existing biodegradable polymers [31&32]. Therefore, in order to increase the strength of a porous ceramic scaffold, polymers for impregnation should have better mechanical properties than the host ceramic. However, another design criterion for polymer-coated bone graft scaffolds requires that the polymer coatings be biodegradable if it is to be used in the regeneration of new tissues and to be able to support the release of pharmaceutical agents and growth factors. This excludes PMMA as a suitable polymer for our “safety glass” coating objective.

As reported in reference [26], Flahiff et al. impregnated coralline hydroxyapatite material with biodegradable PLA. In this study the un-impregnated ceramic was found to have a tensile strength (0.82 MPa), i.e. less than half that of the impregnated scaffold (1.84 MPa). The volume fraction of PLA was equal or larger than that of the ceramic scaffold. The higher volume fraction of a polymer for impregnation results in a greater improvement in the mechanical properties of the porous scaffold with a defined porous structure. Biodegradable PCL has poorer mechanical strength but better toughness than PLA [30&31]. Therefore, it was a natural result that PCL coatings on internal surfaces of porous ceramic scaffold with a smaller polymer fraction would not cause the compressive strength of a scaffold to be increased, as seen clearly in the present study, Figure 10. To coat all the internal surfaces of a scaffold rather than entirely fill the scaffold is supposed to benefit both new bone ingrowth into pores and the fast exposure of biodegradable ceramic scaffold to biological cellular fluids so that the scaffold can participate in the remodeling process as soon as possible.

Another outstanding contribution of PCL coating is that the fractured scaffolds did not deposit and discharge particles during failure under compressive testing, Figure 9. The load-displacement curve obtained in the compressive testing of the scaffold coated with thick PCL coatings (5 coats) had three distinct regions as shown in Figure 10c. In the Region 1, the crushing of pores occurred quickly in the moment the failure happened. The plateau of Region 2 corresponds to the energy absorption during the further crushing of the porous structure. Void space in the porous structure was consumed by the ceramic/polymer composites being compressed. When a significant fraction of struts had been crushed, further increase in the load resulted in the densification of scaffolds and was visible as an upward trend in the curve. There was no further disintegration of scaffolds after prolonged compression. These failure features of the thick PCL coated scaffolds were similar to those of syntactic foam with higher aspect ratio (specimen height/width ratio) that was made by the mechanical mixing of hollow glass spheres in Epoxy resin matrix [33&34]. In syntactic foam, the decrease in the load at the yield point was of the order of about 15 to 20% of the peak load. However here, the decrease at the end of region 1 after failure was about 75% of the peak load in the thickly PCL coated scaffold, Figure 10c. The decrease of compressive load was of almost 100% of the peak load in scaffolds uncoated and with thin PCL coatings, Figure 10(a, b). It is presumed that the processes occurring in the thick PCL-coated scaffolds during compressive testing may also occur when coated scaffolds are applied in clinical situations, i.e. fractured components or particles that will inevitably be generated during shaping or fixation under load-bearing conditions will be held immobile by the polymer until disposed of by the remodeling which occurs during normal bone regeneration.

Conclusions:-

The use of polycaprolactone in conjunction with porous scaffold appears to have merit in providing a suitably strong and coherent material for bone implant clinical procedures, even involving mechanical fixation techniques. In addition, it has the benefit of being available to provide growth factors or other pharmaceutical agents to the trauma site, only releasing them as remodeling occurs.

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