A new resorbable integrated cervical plate/cage fusion device modified with osteoconductive coating or osteoinductive factors: preliminary results in a pre-clinical Yucatan minipig model

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Abstract

Object:

The first goal of this study was to engineer a new resorbable plate/cage construct with a porous cage body capable of withstanding cervical spine loads. The second goal was to test how well the new design supported cervical fusion in a large pre-clinical model up to 18 months using the construct alone, with osteoconductive coating and when delivering rhBMP-7.

Methods:

A cervical plate with integrated porous cage was designed using image-based methods directly from a minipig cervical spine CT. The resulting design was manufactured from a bioresorbable polymer (PCL) using a laser sintering method. The cage portion alone was mechanically tested in compression. An ACDF was performed in 6-9 month old Yucatan Minipigs with one of three experimental groups. One group was the cage/plate construct alone. The second group was the construct coated with Calcium Phosphate (CaP) using a biomineralization technique. The third group was the construct containing a lyophilized collagen sponge with 1.5 mg of rhBMP-7.

Results:

The cage alone exhibited a compressive yield load of 1608 ± 20 N and an effective modulus of 95.0 ± 3.6 MPa. The yield load was much greater than typical human cervical spine loads (~150-200N) while the effective modulus was in the range of human vertebral trabecular bone modulus of 20-100 MPa.

In vivo, all devices retained disc height over the experimental period of 18 months. The CaP coated and rhBMP-7 experimental groups showed more bone formation than the PCL alone. Interestingly, the CaP group demonstrated as much bone growth as the rhBMP-7 group. The PCL material lost 7% molecular weight by 6 months, 22% by 12 months and 35% by 18 months. There was no appreciable difference in material degradation between the three groups

Conclusion:

The newly designed cage/plate cervical fusion construct could provide sufficient mechanical load bearing to support typical cervical spine loads and maintain disc height through the entire 18 months experiment. The CaP coated cage results suggest that use of an osteoconductive coating can significantly increase fusion rates for bioresorbable polymer cages with or without osteoinductive factors. The ability to integrate osteoconductive coating and/or osteoinductive factors like BMP directly with a bioresorbable polymer surface can allow new fusion devices that better distribute and control release of osteoinductive factors in 3D space while allowing better distribution of stresses at the bone/device interface to reduce subsidence and stress shielding.

Introduction:

Anterior Cervical Discectomy and Fusion (ACDF), utilizing synthetic containment plates with either synthetic interbody cages plus bone grafts/osteoinductive factors or femoral dowels has demonstrated success in reducing pain and restoring activity. Fusion rates in ACDF, especially when a cervical plate is utilized, have been reported over 95% [7]. However, despite these high fusion rates, a number of factors have detracted from the success of ACDF. Prominent among these factors are 1) the lack of an osteoinductive factor clearly superior in achieving fusion, and 2) mechanical/material design of the interbody cage leading to subsidence, early implant breakdown and stress shielding. Indeed, these two factors are linked as the choice of osteoinductive factor (particulate autograft versus BMP2) dictates cage design, for example as the need to accommodate bone graft is the origin of the box and cylindrical cage design. Inherently, increasing fusion success will depend on the ability to design fusion systems simultaneously accounting for the osteoinductive/osteoconductive bone growth requirements with the mechanical demands.

The first half of the design problem is determining the osteoinductive factor used to initiate bone growth and ultimately fuse the vertebrae. To date, there is no osteoinductive factor clearly superior in achieving fusion without drawbacks. Iliac crest autograft is considered as the gold standard, but often entails significant pain and morbidity at the harvest site, in some cases leading to ambulation difficulties [21, 29]. Allograft bone dowels are widely used, but can have significantly long remodeling times and exhibit subsidence. Finally, BMP2 use in ACDF has led to significant complications including airway compression and dysphagia that appear to be dose related. The ability to decrease overall dose and control release over a longer period of time would appear beneficial.

A second significant factor detracting from ACDF success is the mechanics of both interbody cages and femoral dowels. Subsidence is noted often in ACDF with reports of up to 60% to 70% incidence [4, 15]. Interbody cage design is a significant risk factor for subsidence [3], with cage designs that have small contact area and increased vertebral bone stress increasing the risk of subsidence [24]. Indeed, Warden and Davy [35] noted increased trabecular tissue damage at the edges of interbody cages, which are locations of high stress concentrations. Furthermore, Vadapalli et al. [34] demonstrated that cages which are excessively stiff compared to vertebral cancellous bone generated much higher vertebral endplate stresses in a finite element study of titanium and PEEK cages. Although clinical effects of subsidence range from unclear to substantial (increased neck pain, instrumentation failure, reduced Odom's scores; [4, 15, 31]), decreasing subsidence is likely to reduce the risks of adverse outcomes.

In addition to subsidence, stress shielding is another interbody cage mechanics/design issue likely to affect fusion outcomes. Stress shielding results when the cage carries a significantly higher proportion of load than ingrown bone tissue. As expected, both material and geometric design factors that increase cage stiffness relative to bone stiffness will increase stress shielding [6, 34]. Although adverse effects of stress shielding are again ambiguous, animal studies have provided evidence that reducing stress shielding can increase the rate and volume of the fusion mass [14, 33].

Although permanent materials (namely PEEK and titanium) provide sufficient mechanical stability for cervical spine fusion, their disadvantages in terms of potential subsidence, long term wear, migration, bacterial colonization and stress shielding have led to investigations of bioresorbable interbody cages. In addition to potential mechanical advantages, the most desirable potential advantage of bioresorbable cages is ability to create a spine fusion that will eventually consist of complete autologously derived bone with no foreign material (either synthetic or allograft) after cage resorption. There are, of course significant challenges with the use of bioresorbable cages: 1) creating mechanically sufficient cages in the first place (given that most bioresorbable materials have inferior strength compared to titanium, PEEK or cortical bone dowels), 2) ensuring that fusion occurs before loss of mechanical stability due to cage resorption, and 3) ensuring that polymer or ceramic degradation products do not have adverse effects on surrounding tissues, for instance osteolysis.

Initial results with bioresorbable cages in both pre-clinical large animal models and clinical studies did indeed support the premise that bioresorbable cages could provide enhanced fusions. For example, Smit et al. compared fusion mass in Poly-l-lactic acid (PLLA) cages to titanium cages (both with iliac crest autograft) at 6 months [33]. They found a faster formation rate and larger fusion mass in the PLLA cage compared to the titanium cage which was attributed to the reduced stiffness of the PLLA cage. However, later clinical results with hydrosorb, 70/30 poly-L-lactide-CO-D,L-lactide (70/30 PLDLLA) bioresorbable cages demonstrated reduced fusion rates, and significantly increased subsidence rates when compared to PEEK cages after 2 years [12]. It was hypothesized that these implants degraded early, losing strength with the degradation products causing osteolysis, which accelerated subsidence. It was noted that the cages, however, were sterilized using E-beam irradiation, which causes significant reductions in molecular weight and subsequent mechanical strength of the cages. Furthermore, it was noted previously by Krijnen et al. in a sheep model that PLDLLA exhibited early cracking and mechanical failure by 3-6 months [16]. The attributed increased pseudoarthrosis rate in the PLDLLA cages to increased motion resulting from cage failure. Lazennec et al. performed lumbar fusion in sheep using 96/4 PLDLLA [18]. They found successful fusion by 9 months with the majority of the implants resorbed by 36 months. Finally, Abbah et al. utilized Polycaprolactone/Tri-calcium phosphate (PCL/TCP) porous scaffolds manufactured using Fused-deposition modeling, a rapid prototyping technique [1]. The PCL/TCP scaffolds were utilized with metal screws and rods to stabilize the spinal segments, and delivered rhBMP2 from lyophilized sponges. Results demonstrated fusion at 6 months, equivalent to iliac crest autograft. An advantage of PCL is that since it is a slowly resorbing material (typically over 2 years), a successful fusion (typically 9 months to 1 year) can be obtained before significant degradation occurs, addressing issues seen by Jiya et al. [12] and Krijnen et al. [16].

It is clear that results with bioresorbable interbody fusion cages have been mixed, due to the challenges in timing the residual strength of the bioresorbable cage with strength development of the fusion mass. Although the reduced stiffness of bioresorbable cages may aid bone growth and fusion, this must be balanced with the need to provide sufficient strength in the fusion space initially and maintain this strength at least 6 to 9 months (perhaps longer) until fusion and stability is achieved. Furthermore, bioresorbable cage design could dramatically decrease subsidence rate due to material stiffness and geometric design, but most bioresorbable cages to date except that of Abbah et al. have mimicked the box or cylinder cage designs of PEEK and titanium [1]. Finally, similar to permanent materials like PEEK and titanium, bioresorbable polymer cages possess no inherent osteoconductivity or osteoinductivity, relying on bone graft or BMP2 release to stimulate bone growth. However, given the great variety of methods for surface modification of bioresorbable polymers, one would expect that bioresorbable polymer cages could be modified with enhanced osteoconductivity.

The design challenges of bioresorbable polymer cages are numerous, but if these challenges can be addressed there is great potential to enhance fusion outcomes with bioresorbable cages. The purpose of the current study was to design and manufacture a cervical interbody bioresorbable fusion case to address these design challenges. Specifically, we designed an interbody cage with 3D pore architecture that had sufficient rigidity and strength to carry *in vivo* human cervical spine loads while having a low modulus and high contact area to avoid subsidence and stress shielding. We also designed an integrated

cervical plate with the cage, simplifying surgical implantation. We then fabricated the designed cage from PCL. We further implemented biomineralization techniques to make a second group of cages osteoconductive with a degradable, integrated Calcium Phosphate (CaP) layer. A third group of cages was made osteoinductive by delivering BMP7 from collagen sponges lyophilized on the porous interbody cage structure. We then determined the mechanical properties of the cages and tested their capability for supporting cervical spine fusion in a pilot pre-clinical large animal study.

Materials and Methods:

The integrated bioresorbable cervical interbody cage/plate constructs were designed using image-based techniques and manufactured from polycaprolactone (PCL) using laser sintering. Three experimental groups were implanted including 1) PCL cage/plate construct alone, 2) PCL cage/plate coated with a resorbable Calcium Phosphate (CaP) layer, and 3) PCL cage/plate construct delivering 1mg of recombinant human bone morphogenetic protein 7 (rhBMP7). Constructs were then implanted in the C5-C6 disc space of 9 month old Yucatan minipigs for 6, 12 and 18 months. Bone fusion was characterized using micro-CT scanning. Cage/plate degradation was characterized by determining molecular weight loss.

Integrated bioresorbable cervical interbody cage/plate constructs were designed using image-based techniques [8-10,22]. A Computed Tomography (CT) scan of a Yucatan Minipig cervical spine was used to define the anterior containment plate and interbody cage design regions. To allow bone ingrowth, a 3D porous architecture was designed to provide maximum stiffness in the Interior-Superior direction to carry in vivo spine loads and in the Anterior-Posterior direction to carry surgical impaction loading while maximizing permeability to allow bone ingrowth for fusion. The resulting pore size was 1.7mm. The containment plate contained holes for screw fixation and additionally contained grooves that slide over vertebral distraction pins for surgical alignment. The resulting voxel designs were converted into .STL format for fabrication.

The base bioresorbable plate/cage construct was manufactured from PCL using a laser sintering technique previously described [28,36]. PCL powder (CAPA 6501, Solvay Caprolactones, UK) was processed on a Selective Laser Sintering (Sinterstation 2000™, 3D Systems, Valencia, CA) with a layer thickness of 100 µm. The final manufactured specimen matched the image-based design.

PCL by itself is neither osteoconductive nor osteoinductive. We addressed both of these issues by performing post-fabrication modification of the base cage/plate construct. In one group, we created an osteoconductive construct by biomineralizing the PCL surface using a Simulated Body Fluid (SBF) method developed by Murphy and colleages for polyester resorbable polymers [26, 27]. To induce formation of a CaP-based mineral layer, constructs were subjected to hydrolysis treatment to hydrolyze ester bonds on the scaffold surface and reveal carboxylic acid groups. These groups have been shown to induce heterogeneous mineral nucleation on degradable polymer substrates. The scaffolds were then incubated in modified simulated body fluid (mSBF) solutions for mineral nucleation and growth. The mSBF solution contains the ionic constituents of blood plasma, with double the concentrations of calcium and phosphate ions, and is held at physiologic temperature and pH 6.8.

To create an osteoinductive scaffold, a type I collagen sponge was first lyophilized into the porous interbody space to serve as a carrier. The sponge within the porous interbody spacer was then soaked with 1.5mg of rhBMP7/40mM Acetic Acid (ProSpec-Tany TechnoGene, Rehovot, Israel) 20 minutes prior to implantation.

Prior to implantation, a group of four interbody cages alone without the integrated containment plate were mechanically tested in compression to determine load carrying capacity. Compression testing was performed on a MTS RT/30 Alliance at a displacement rate = 0.1 mm/min to determine the effective modulus and yield load of the cage region. Micro-computed tomography (µCT) was completed on a subset of the complete integrated plate/cage structures to assess manufacturing quality.

An ACDF procedure was performed on 6-8 month old Yucatan mini-pigs (13 total) at the C5-C6 level (Fig. 1). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals. This experiment was set up as pilot study to assess the long term load bearing, fusion

and degradation of this bioresorbable cervical system, with and without osteodonductive and osteoinductive modification. We had three experimental groups: 1) PCL construct alone, 2) PCL with CaP coating, and 3) PCL loaded with rhBMP-7. Time points for sacrifice were 6, 12 and 18 months. The complete experimental layout giving the number of animals per time point and treatment are shown in Table 1.

Prior to sacrifice, CT scans were completed on anesthetized animals to assess bone ingrowth. After sacrifice at 6, 12, or 18 months, the fusions were further assessed with μCT (MS-130, GE Medical Systems, Toronto, CAN). Bone ingrowth was calculated from micro-CT as the volume of mineralized tissue within the interbody pore space divided by the total interbody pore space volume.

Degree of polymer biodegradation was determined by changes in average molecular weight and molecular number. Specially, samples of PCL from each fusion cage were removed after sacrifice at 6,12, or 18 months, dissolved in tetrahydroflouran (THF) and subjected to Gas Permeability Chromotography (GPC) analysis to determine the average molecular weight (Mw) and molecular number (Mn). Polydispersity, a measure of polymer chain heterogeneity, as calculated as molecular weight divided by molecular number (Mw/Mn).

Results:

The manufactured cage/plate construct matched the designed construct (Fig. 2). Micro-CT scanning demonstrated dense material with little manufacturing defects (Fig. 2c). The interbody cage alone in compression testing had a yield load of 1608 ± 20 Newtons (N) and an effective modulus of 95.0 ± 3.6 MPa. The standard deviation of the yield load was 1.2% of the mean and that of the effective modulus as 3.8% of the mean.

In Vivo CT scans demonstrated a progressively increased bone growth within the fusion body for the CaP coated group (osteoconductive modification) as well as the rhBMP-7 group (osteoinductive modification) (Fig. 2). The cages maintained disc space and bone grew through the porous interbody architecture to bridge the inferior and superior vertebrae as determined from both *in vivo* CT scans and micro-CT (Fig. 3). Micro-CT calculations of percent bone ingrowth in the interbody portion confirmed the *in vivo* CT result at 6, 12, and 18 months (Fig. 4). While both the CaP coated group and rhBMP-7 group demonstrated increasing bone formation with time, the uncoated PCL group maintained relatively constant bone ingrowth over time. The CaP coated cage/plate constructs had similar amounts of bone ingrowth to the plate/cage constructs delivering rhBMP-7.

GPC results for molecular weight demonstrated the slow degradation that is characteristic of PCL. Pooling all groups demonstrated showed a 7% decrease in average molecular weight at 6 months, a 24% decrease at 12 months, and a 39% decrease after 18 months (Fig. 5). Results for average molecular number were similar (5% at 6 months, 22% at 12 months, and 35% at 18 months; Fig. 6). Polydispersity decreased with time *in vivo*. The molecular weights of the as received PCL powder and that after laser sintering were equivalent, demonstrating that the manufacturing process did not significantly change the material composition. Molecular weight decreases for uncoated PCL, CaP coated PCL and collagen lypholized rhBMP-7 PCL were equivalent at all time points (Fig. 6).

Discussion:

Although limitations of this study do to the number of animals tested did not permit statistical testing between groups, the current study did demonstrate that the newly designed bioresorbable PCL interbody plate/cage constructs could maintain cervical disc space and support bony fusion. The osteoconductive (CaP coated) and osteoinductive cages (rhBMP-7) had more bone ingrowth then the uncoated PCL cages, although some level of bone growth was seen in the PCL cages. The need to enhance the osteoconductivity and/or osteoinductivity of PCL is not surprising. Abbas et al. (2008) also found that bone fusion in a minipig lumbar spine fusion model could only be reliably obtained when delivering rhBMP-2 compared to an uncoated PCL cage that was neither osteoconductive or osteoinductive.

An interesting result is that a purely osteoconductive scaffold modification (CaP coating) led to results in this pilot study equivalent to delivery of rhBMP-7 delivery from lyophilized type I collagen sponge in an osteoinductive scaffold. Of course, a larger pre-clinical animal model study is needed to determine how well a purely osteoconductive scaffold can support bone growth and fusion compared to an osteoinductive scaffold. In addition, the efficacy of rhBMP-7 as osteoinductive factor compared to rhBMP-2 has been questioned [2]. However, the initial data reported here showing initial equivalence clearly demonstrate that a study comparing a purely osteoconductive approach (CaP) coating compared to an osteoinductive approach is worthy to pursue. Indeed, the ability to achieve fusion with no or limited osteoinductive factors or bone graft would represent a significant advance in fusion techniques. Further testing to determine the amount of BMP delivered with this model would be helpful, as well as studies to determine the drug carrying capabilities of an osteoconductive implant with BMP incorporated directly into the coating [13].

In these regards and with regard to osteoconductivity and osteoinductivity, the current PCL material and manufacturing approach provides a number of advantages over currently used cervical interbody fusion materials. First, the PCL material itself can readily be modified with CaP coating to create an osteoconductive scaffold. This CaP coating is degradable, allowing creation of a totally resorbable load bearing fusion system. Furthermore, the CaP coating can readily bind proteins like BMP2 and BMP7 [13], allowing quick binding in the Operating Room (OR) to create an osteoinductive and osteoconductive scaffold that is completely resorbable. Although titanium can be modified with HA or even a resorbable HA, it remains of course a permanent material with associated risk of stress shielding and loosening. Likewise, PEEK remains a permanent material and itself is not an osteoconductive material. Furthermore, no studies have been presented demonstrating the ability to readily manufacture a porous interbody cage integrated with a containment plate using PEEK or titanium.

A significant concern with using resorbable materials is their ability to carry load both initially and as the material resorbs. If such a material resorbs too quickly before bone formation, there is a risk disc space collapse due to material failure. Indeed, such a scenario was seen for 70/30 PDLLA by Krijnen et al. in lumbar spine fusion [16]. They noted that the material exhibited increased cracking and brittle behavior in a sheep model between 3 and 6 months. Jiya et al. found similar results in a clinical study, further attributing osteolysis and increased rates of pseudoarthrosis to premature degradation and loss of mechanical integrity [12]. Such results may be related to the combination amorphous/crystalline make-up of the polylactic acid polymers. As such polymers degrade, the amorphous regions degrade first, increasing the crystallinity of the remaining polymer. The resulting molecular weight loss decreases the polymer mechanical strength, while the increasing crystallinity may increase the likelihood of brittle failure and cracking. An advantage of PCL over the polylactic acids is that PCL is an amorphous polymer that exhibits significant ductility (post-yield deformation). Partee et al. [28] showed that the post-yield deformation of laser sintered PCL was over twice that of pre-yield deformation, demonstrating extensive ductility. Our own results (Kang et al., unpublished data) demonstrated that lumbar spine cages in compression could undergo 7% strain before yielding and up to 50% strain without fracture. Our current results showed that the designed porous interbody cages could withstand over 1600 N of load before yielding. This load level is well beyond the typical cervical spine loads of 150-200 N [23, 25]. Indeed it has been demonstrated that human cadaver cervical spine segments begin to demonstrate damage at 300 N compressive loads [30]. Our designed PCL cervical cages can therefore readily carry cervical spine loads without failure. This was verified *in vivo* up to 18 months by CT and micro-CT scans showing that disc height was maintained. Furthermore, the bone ingrowth patterned followed the designed porous architecture, which would not have been seen if the cage would have mechanically failed and the architecture would have significantly deformed.

Withstanding cervical loads without failure, however, is only one aspect of a properly mechanical functioning cage. Another aspect is carrying these loads without stress shielding the ingrown bone or engendering high stresses at the cage trabecular bone interface that increase cage subsidence. Both stress shielding and subsidence have been shown to increase with increasing cage stiffness [33]. Smit et al. noted that resorbable 70/30 Poly(l,dl-lactide) (70/30 PDLLA) cages showed a significantly greater fusion mass

in goats compared to much stiffer titanium cages after 3 years, demonstrating the adverse impact of stress shielding on fusion mass [33].

One of the most significant potential advantages of the PCL cage/plate construct is the ability to integrate structural support with osteoinductive factor delivery. Current approaches use a separate structural cage made from PEEK or titanium with a separate delivery vehicle, typically a collagen sponge. However, it is precisely the need to hold a separate volume of osteoinductive material that gives rise to the box or open cylinder geometry known as a cage. This cage design geometry leads to higher stresses at the cage bone interface [24]. These higher interface stresses lead to damaged trabeculae and also increase the rate of subsidence [35]. The ability to distribute osteoconductive factors like CaP coating and use either CaP coating [5,13,19,20] or direct conjugation [11,36] to distribute osteoinductive factors (both protein and gene-based therapies) directly on the structural surface provides a new design approach for spine fusion. Specifically, freed from the need to have a box cage design for delivering separate osteoinductive factors, the porous mesh cage in this study distributes stresses more evenly at the cage bone interface. Thus, an integrated structural delivery vehicle would be an advantage for both osteoinductive factor delivery and better load distribution to reduce stress shielding and subsidence.

In conclusion, we have presented a new bioresorbable integrated plate/cage construct for cervical spine fusion. This new construct was further modified to be osteoconductive with a CaP coating or osteoinductive with a collagen sponge delivering rhBMP-7. The new device supported yield loads (1600N) that were much greater than typical cervical loads (~150-200N), and further was able to maintain disc height *in vivo* for the entire 18 month experimental period. The CaP coated scaffolds provided bone growth and fusion equivalent to the rhBMP-7 cage. Since this CaP coating can also be used to delivery BMPs, this result raises the potential of an integrated structural delivery vehicle that can obtain spine fusion with much lower BMP doses than current approaches. Finally, the capability to integrate osteoconductive/osteoinductive factors directly on a resorbable material substrate allows a wider range of porous cage designs that can reduce stress shielding and subsidence.

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References

1) Abbah SA, Lam CX, Hutmacher DW, Goh JC, Wong HK: Biological performance of a polycaprolactone-based scaffold used as fusion cage device in a large animal model of spinal reconstructive surgery. **Biomaterials 30:**5086-5093, 2009

2) Agarwal R, Williams K, Umscheid CA, Welch WC: Osteoinductive bone graft substitutes for lumbar fusion: A systematic review. **J Neurosurg Spine 11:**729-740, 2009

3) Barsa P, Suchomel P: Factors affecting sagittal malalignment due to cage subsidence in standalone cage assisted anterior cervical fusion. **Eur Spine J 16:**1395-1400, 2007

4) Chen Y, Chen D, Guo Y, Wang X, Lu X, He Z, et al: Subsidence of titanium mesh cage: A study based on 300 cases. **J Spinal Disord Tech 21:**489-492, 2008

5) Choi SY, Murphy WL. Sustained plasmid DNA release from dissolving mineral coatings. **Acta Biomaterialia**, 6: 3426-3435, 2010.

6) Epari DR, Kandziora F, Duda GN: Stress shielding in box and cylinder cervical interbody fusion cage designs. **Spine (Phila Pa 1976) 30:**908-914, 2005

7) Fraser JF, Hartl R: Anterior approaches to fusion of the cervical spine: A meta-analysis of fusion rates. **J Neurosurg Spine 6:**298-303, 2007: 1

8) Hollister SJ, Levy RA, Chu TM, Halloran JW, Feinberg SE: An image-based approach for designing and manufacturing craniofacial scaffolds. **Int J Oral Maxillofac Surg 29:**67-71, 2000

9) Hollister SJ, Maddox RD, Taboas JM: Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. **Biomaterials 23:**4095-4103, 2002

10) Hollister SJ: Porous scaffold design for tissue engineering. **Nat Mater 4:**518-524, 2005

11) Hu WW, Elkasabi Y, Chen HY, Zhang Y, LaHann J, Hollister SJ, Krebsbach PH: The use of reactive polymer coatings to facilitate gene delivery from poly (e-caprolactone) scaffolds. **Biomaterials**, 30:5785-5792, 2009.

12) Jiya T, Smit T, Deddens J, Mullender M: Posterior lumbar interbody fusion using nonresorbable poly-ether-ether-ketone versus resorbable poly-L-lactide-co-D,L-lactide fusion devices: A prospective, randomized study to assess fusion and clinical outcome. **Spine (Phila Pa 1976) 34:**233-237, 2009

13) Jongpaiboonkit L, Franklin-Ford T, Murphy WL. Mineral-coated, biodegradable microspheres for controlled protein binding and release, **Advanced Materials**, 21:1960–1963, 2009

14) Kandziora F, Schollmeier G, Scholz M, Schaefer J, Scholz A, Schmidmaier G, et al: Influence of cage design on interbody fusion in a sheep cervical spine model. **J Neurosurg 96:**321-332, 2002

15) Kast E, Derakhshani S, Bothmann M, Oberle J: Subsidence after anterior cervical inter-body fusion. A randomized prospective clinical trial. **Neurosurg Rev 32:**207-14; discussion 214, 2009

16) Krijnen MR, Mullender MG, Smit TH, Everts V, Wuisman PI: Radiographic, histologic, and chemical evaluation of bioresorbable 70/30 poly-L-lactide-CO-D, L-lactide interbody fusion cages in a goat model. **Spine (Phila Pa 1976) 31:**1559-1567, 2006

17) Lam CX, Hutmacher DW, Schantz JT, Woodruff MA, Teoh SH: Evaluation of polycaprolactone scaffold degradation for 6 months in vitro and in vivo. **J Biomed Mater Res A 90:**906-919, 2009

18) Lazennec JY, Madi A, Rousseau MA, Roger B, Saillant G: Evaluation of the 96/4 PLDLLA polymer resorbable lumbar interbody cage in a long term animal model. **Eur Spine J 15:**1545-1553, 2006

19) Lee JS, Lee JS, Murphy WL. Modular peptides promote human mesenchymal stem cell differentiation on biomaterial surfaces, **Acta Biomaterialia**, 6: 21-28, 2010.

20) Lee JS, Lu Y, Baer GS, Markel MD, Murphy WL. Controllable protein delivery from coated surgical sutures. **Journal of Materials Chemistry**, In Press, 2010.

21) Lied B, Roenning PA, Sundseth J, Helseth E: Anterior cervical discectomy with fusion in patients with cervical disc degeneration: A prospective outcome study of 258 patients (181 fused with autologous bone graft and 77 fused with a PEEK cage). **BMC Surg 10:**10, 2010

22) Lin CY, Kikuchi N, Hollister SJ: A novel method for biomaterial scaffold internal architecture design to match bone elastic properties with desired porosity. **J Biomech 37:**623-636, 2004

23) Lin CY, Kang H, Rouleau JP, Hollister SJ, LaMarca F: Stress analysis of the interface between cervical vertebrae end plates and the bryan, prestige LP, and ProDisc-C cervical disc prostheses: An in vivo image-based finite element study. **Spine (Phila Pa 1976) 34:**1554-1560, 2009

24) Lowe TG, Hashim S, Wilson LA, O'Brien MF, Smith DA, Diekmann MJ, et al: A biomechanical study of regional endplate strength and cage morphology as it relates to structural interbody support. **Spine (Phila Pa 1976) 29:**2389-2394, 2004

25) Moroney SP, Schultz AB, Miller JA, Andersson GB: Load-displacement properties of lower cervical spine motion segments. **J Biomech 21:**769-779, 1988

26) Murphy WL, Kohn DH, Mooney DJ: Growth of continuous bonelike mineral within porous poly(lactide-co-glycolide) scaffolds in vitro. **J Biomed Mater Res 50:**50-58, 2000

27) Murphy WL, Mooney DJ: Bioinspired growth of crystalline carbonate apatite on biodegradable polymer substrata. **J Am Chem Soc 124:**1910-1917, 2002

28) Partee B, Hollister SJ, Das S: Selective laser sintering process optimization for layered manufacturing of CAPA 6501 polycaprolactone bone tissue engineering scaffolds. **ASME J. Man. Sci Eng., 128**:531-540.

29) Pollock R, Alcelik I, Bhatia C, Chuter G, Lingutla K, Budithi C, et al: Donor site morbidity following iliac crest bone harvesting for cervical fusion: A comparison between minimally invasive and open techniques. **Eur Spine J 17:**845-852, 2008

30) Przybyla AS, Skrzypiec D, Pollintine P, Dolan P, Adams MA: Strength of the cervical spine in compression and bending. **Spine (Phila Pa 1976) 32:**1612-1620, 2007

31) Schmieder K, Wolzik-Grossmann M, Pechlivanis I, Engelhardt M, Scholz M, Harders A: Subsidence of the wing titanium cage after anterior cervical interbody fusion: 2-year follow-up study. **J Neurosurg Spine 4:**447-453, 2006

32) Silber JS, Anderson DG, Daffner SD, Brislin BT, Leland JM, Hilibrand AS, et al: Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. **Spine (Phila Pa 1976) 28:**134-139, 2003

33) Smit TH, Muller R, van Dijk M, Wuisman PI: Changes in bone architecture during spinal fusion: Three years follow-up and the role of cage stiffness. **Spine (Phila Pa 1976) 28:**1802-8; discussion 1809, 2003

34) Vadapalli S, Sairyo K, Goel VK, Robon M, Biyani A, Khandha A, et al: Biomechanical rationale for using polyetheretherketone (PEEK) spacers for lumbar interbody fusion-A finite element study. **Spine (Phila Pa 1976) 31:**E992-8, 2006

35) Warden KE, Davy DT: Localized trabecular damage adjacent to interbody fusion devices. **Spine (Phila Pa 1976) 35:**874-880, 2010

36) Williams JM, Adewunmi A, Schek RM, Flanagan CL, Krebsbach PH, Feinberg SE, Hollister SJ, Das S: Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. **Biomaterials 26**:4817-4827.

37) Zhang H, Migneco F, Hollister SJ: Bone morphogenetic protein-2 conjugated on three-dimensional polycaprolactone (PCL) scaffolds stimulates osteogenic activity in bone marrow stromal cells. **Tissue Eng A**, in press.