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Design and characterization of poly-ε-caprolactone electrospun fibers incorporated with α-TCP nanopowder as a potential guided bone regeneration membrane

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Abstract

Periodontitis can lead to the destruction of oral mucosa. Therefore, in order to improve tissue/bone regeneration, it is important to facilitate the process of attachment and proliferation of the cells. Considerable efforts had been devoted toward the development of electrospun membranes, as guided bone regeneration (GBR), which are based on poly (ε -caprolactone) (PCL). However, most of previous membranes have lacked the structural and mechanical strength and toughness to engineer bone tissue constructs with suitable biocompatibility functions. Here, we developed bioactive and relatively robust hybrid membranes composed of α-tricalcium phosphate (α-TCP) nanopowder embedded PCL electrospun fibers. Incorporation of various concentrations of α-TCP nanopowder from 0 to 2 wt % within the PCL membranes notably improved tensile strength and toughness. In a nutshell we concluded that PCL-α-TCP membranes could potentially be used to develop clinically relevant constructs for bone tissue engineering.

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Keywords: Guided bone regeneration; Polycaprolactone; α-tricalcium phosphate; mechanical properies

1. Introduction

Inflammatory disease such as Periodontitis can lead to the destruction of oral mucosa. Therefore, in order to improve tissue/bone regeneration it is important to facilitate the process of attachment and proliferation of the cells. Among various

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methods to regenerate tissue/bone, a membrane technique was used to generate bone around the implants. This technique is one of the most promising augmentation techniques which covers bone defects and encourages new bone ingrowth while at the same time it prevents the ingrowth of fibrous tissue into the grafted site [1, 2]. GBR membranes are not only used to perform the barrier function by preventing the ingrowth of fibroblast cells into the tissue/bone defect site but also are used to improve the tissue/bone regeneration by supporting cells to attach and proliferate. These membranes are placed between the soft tissue and the regenerating bone to prevent the gingival tissues from intruding into the alveolar bone site [3, 4]. An ideal membrane for GBR application need to have biocompatibility, proper degradation rate, high interconnected porosity to be cell occlusive, fibrous structure to mimic natural fibrous structures in the extracellular matrix and adequate mechanical and physical properties [4]. Moreover, GBR membrane should help cell exclusion to separate the gingival flap from the fibrin clot and to guard space for the new alveolar bone and the periodontal ligament [5]. The pore size of electrospun membranes for GBR application in general is less than the average cell size, and previous studies have shown that such small pores do not allow cell penetration [1].

Several biodegradable polymers such as poly (L-lactic acid) (PLA) [6], Poly (Lactic-Co-Glycolic Acid) (PLGA) [7], and Poly(caprolactone) (PCL) [8] are widely used in bone tissue engineering. Among these polymers PCL as a semi crystalline biodegradable synthesis polymer has many features such as slow biodegradability which can protect GBR site long enough for complete healing, good biocompatibility, high drug permeability, and better mechanical property for resisting the mechanical stress caused by the surgical operation and the tissue of operation zone, which make it a good candidate material for biomedical applications. Another advantage of PCL is that this polymer does not produce a local acidic environment as it degrades which could lead to an acidosis [9]. Although present polymeric products show positive results in clinical studies, their weak mechanical properties and poor bone regeneration capacity are still major challenges.

To overcome these problems, recent research efforts have included the incorporation of bone-like ceramics into the membranes, e.g. hydroxyapatite [1], tricalcium phosphate (TCP) [10] and calcium carbonate [1]. In this regard, due to the bioactivity and osteoconductivity of calcium phosphate as well as similarity to mineral phase of bone, these materials have been widely used as bone regenerator [11]. TCP ceramics have two polymorphous which are stable at high and low temperature known as α-TCP and β-TCP, respectively. HA and Ca-P are different in their crystalline structure and absorption characteristics. Depending on their forms and chemical composition, Ca-P have a higher resorption than HA. Therefore, both chemical dissolution and cell-mediated resorption were involved in the resorption process. Moreover, α-TCP has higher free energy and a lower density than other reactants such as beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA). due to the presence of atomic vacancies in α-TCP structure and its high resorption leading to be used extensively as constituent of GBR membrane [12].

The aim of the present study is to improve bioactivity, degradability, and mechanical characteristics through incorporation of α-TCP nanopowder within PCL electrospun fibrous membranes. It is hypothesized that the development of a bioactive and mechanical robust fibrous membrane could simultaneously induce proper cues such as chemical, topographical and mechanical cues to support bone cell functions.

2. Materials and method

PCL (Mw=80,000) was purchased from Sigma–Aldrich, USA. Chloroform and methanol were also obtained from Merck chemicals, Germany. Moreover, for synthesized α-TCP nanopowder, Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), phosphorus pentoxide (P_2O_5), amorphous silica (SiO_2) and disodium hydrogen phosphate (Na_2HPO_4) were provided from Merck, Germany.

α-TCP nanopowder was synthesized according to the previous research [13], briefly, Si-stabilized α-TCP was synthesized in a two-step process of sol-gel and mechanical alloying. Primarily, calcium deficient hydroxyapatite (CDHA) was synthesized and calcinated at 650 ºC for 1 hr. CDHA was mixed with amorphous silica and dried for 24 hr at 110 ºC and the resultant powder was sintered at 1250 ºC. Following, synthesized α-TCP nanopowder was milled in a planetary ball mill (Restsch PM100, Germany) using four agate balls (diameter 15 mm) at 250 rpm for 3hr in order to prevent agglomeration.

Nanocomposite fibrous membrane with different amounts of α-TCP nanopowder (0–2 wt.%) were prepared by electrospinning method. PCL pellets were dissolved in chloroform: methanol (4:1 (v/v) %) with a concentration of

10 wt.% and mixed with α-TCP nanopowder with various concentrations. α-TCP nanopowder is completely dispersed and sonicated for 30 min (WUDD10H, Power 770 W). For developing the intended fibrous membrane, as prepared suspensions, which was fed into 1 mL syringe having a 23G blunted stainless steel needle was electrospun. As prepared α-TCP:PCL fibrous membrane were labeled based on α-TCP concentration (0.5, 1, and 2 wt.%) as 0.5α-PCL, 1α-PCL, and 2α-PCL, respectively.

2.1 Characterization of α-TCP/PCL fibrous membrane

The phase composition of the α-TCP was assessed by X-ray powder diffraction (XRD, X'Pert Pro X-ray diffractometer, Phillips, Netherlands). XRD was performed with CuKα radiation (λ=0.154nm, 40 kV, 40 mA), data collected with the step size = 0.02 and set time = 1.5s.

The surface morphology of prepared fibrous membranes was studied by scanning electron microscope (SEM, Philips, XL30). The samples were sputter coated with a thin layer of gold before analysis. Moreover, the fiber size and pore size of the electrospun fibrous membranes were also determined (n=30) using SEM images along with NIH Image J software.

The chemical characteristics of fibrous membranes were investigated by using Fourier-transform infrared (FTIR) spectroscopy (Bomem, MB 100) performed over a range of 400-4000 cm-1 and resolution of 2 cm^{-1}.

The mechanical properties of membranes were determined by using uniaxial tensile testing technique (Instron, Model 5542) with a 10 N load cell and at a cross-head speed of 2 mm/min. Rectangular samples (length of 40–50 mm, width of 9-10 mm and thickness of 0.3–0.5 mm) (n = 5) were prepared and the uniaxial tensile properties were measured.

2.2 Statistical analysis

One-way ANOVA (n≥3) was applied to establish a statistical significance difference between groups, Tukey's post-hoc test using GraphPad Prism Software (Version 6) with a p-value <0.05 was considered to be significant.

3. Results and discussion

To synthesize α-TCP nanopowder, a two-step process consisting of synthesizing of CDHA by sol-gel method followed by Si substitution of CDHA via mechanical alloying was used. XRD patterns of α-TCP calcified at 1250 ºC (Fig. 1) demonstrate that powder consisted of pure α-TCP and confirmed the formation of well crystalline α-TCP powder. Peaks at around 30.7 and 34.2 correspond to the α-TCP crystal planes of (034) and (290), respectively. The role of Si was noticeable since no peaks in relation to β-TCP or HA were revealed. So, incorporation of Si element to TCP structure prevented the lather from phase transition during cooling and the α-TCP phase remain stable. As demonstrated in other researches, to obtain α-TCP powder, it was necessary to quench the samples [14,15], but the addition of Si yields in retained α-TCP upon slow cooling rate [16,17].

The core focus of this study was to fabricate α-TCP:PCL fibrous membrane using electrospinning technique. In order to generate bone tissue, α-TCP:PCL fibrous scaffolds were fabricated using electrospinning technique. Fig. 2 shows the SEM images of α-TCP:PCL fibrous membranes consisting of various amount of α-TCP nanopowder (0.5, 1, and 2 wt.%). Addition of α-TCP nanopowder to PCL solution resulted in increased surface tension and viscosity of solution leading to increase fiber size and pore size of scaffolds to be precise, by adding α-TCP nanopowder the fiber size increased from 554.5±48.8 nm (for PCL) to 434.3±67.6 nm, 577.8±110.8 nm, and 801.3±112.9 nm (For PCL-0.5α, PCL-1α, and PCL-2α, respectively) ($p<0.05$). In the other research, it was demonstrated that by adding Gr nanoparticles the fiber diameter increased after adding 2 and 5 wt% Gr which is due to the changes in viscosity of polymeric solution [18]. Moreover, the pore size of fibrous membranes was changed significantly for PCL (2.74±0.39 μm) and PCL-1α (1.86±0.42 μm) fibrous membrane ($p<0.05$).

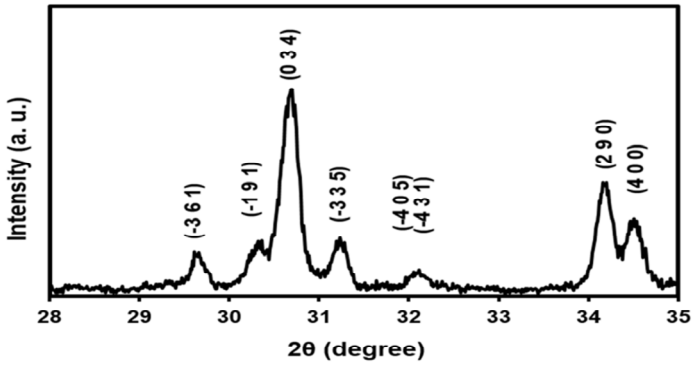

Fig. 1. XRD pattern of α -TCP nanopowder.

Due to the formation of fibrous membranes consisting of uniform architecture and smooth fibers with appropriate sizes, PCL-1α mixture was selected for further experiments. Such results demonstrated in other researches which by incorporation forsterite, the average fiber size increased significantly and the surfaces of fibers were turned rougher [19]. Similar results were reported in other research which the pore size of fibrous membrane decreased as the HA volume fraction increases [20]. In another research, the addition of diopside nanoparticles up to 7 wt.% yields in decrease in pore size of PCL scaffolds and reached to 9.5±1.1 μm (for 7 wt.% diopside) from 38.6±3.7 μm. This is due to the fact that with increasing α-TCP content, the fiber diameter reduced and more layers of fibers overlapped with each other to fill the pores and decrease the pore size [21,22].

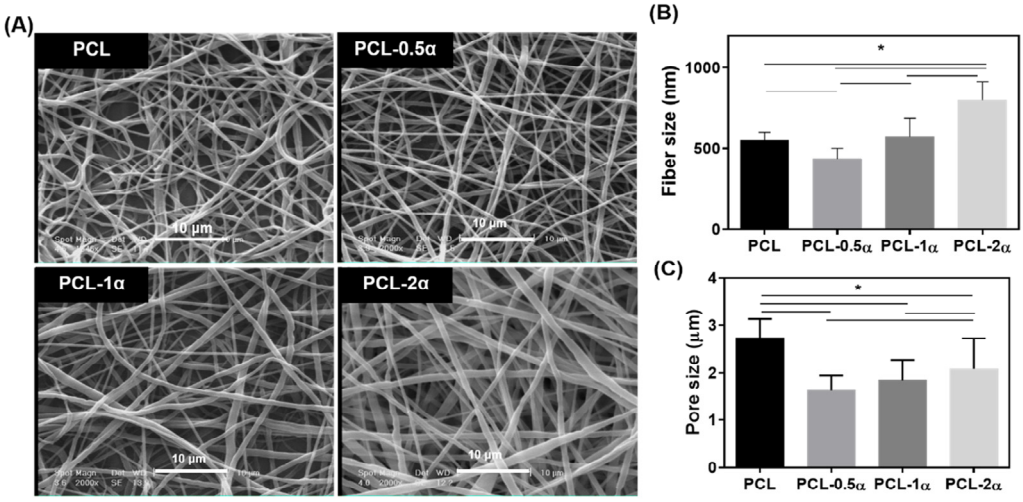

Fig.2. Structural and physical properties of α -TCP:PCL fibrous membranes; (A) SEM images of fibrous scaffold, (B) Fiber size and (C) pore size of fibrous membranes.

To evaluate the chemical properties of fibrous membranes, FTIR analysis as a powerful method to identify the functional groups was applied. According to Fig. 3, the FTIR spectrum of Si-stabilized α-TCP nanopowder revealed the strongest bands in the range of 570-620 cm^{-1} (triply degenerate P–O asymmetric bending) and 940-1230 cm^{-1} (P–O) corresponded to the vibrations of phosphate groups. Si-O bands were located at 470 and 800 cm^{-1} which

confirmed the substitution of Si within α-TCP via sol-gel and mechanochemical processes which is in accordance with other researches [23, 24]. Furthermore, PCL fibrous membranes consisted of the main characteristic peaks of PCL at 1726 cm^{-1} (stretching vibrations of the carboxyl (C-O)) and 1180 cm^{-1} (stretching vibrations of the ether groups (C-O-C)). Similar results were demonstrated in other researches [25]. According to the spectra of α-TCP:PCL fibrous membrane, there are some peaks (1724cm^{-1}) which are shifted to lower wave number, which demonstrated that there are some interaction between PCL and α-TCP. These interactions are hydrogen bonding which can affect the other properties such as mechanical properties. Such similar results demonstrated in other researches which revealed that interaction of polymer and nanoparticles affected the mechanical properties [18, 26]. Similar results were revealed in other researches that the position of characteristics peaks related to phosphate group vibrations did not affected after the incorporation of Si in the crystal lattice [11].

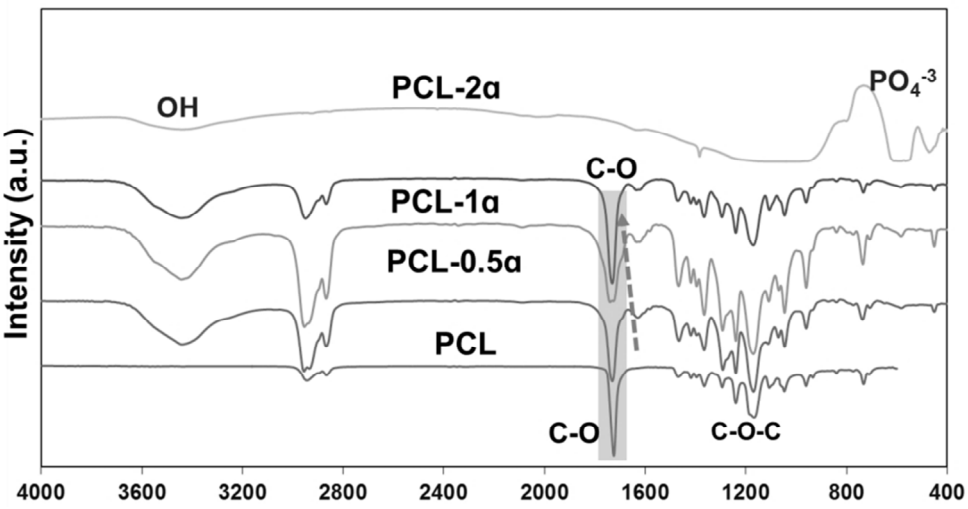

Fig. 3. FTIR spectra of the fibrous scaffolds.

In addition to chemical properties, the mechanical properties of the scaffolds were investigated by uniaxial tensile test. The typical stress-strain curves and detailed mechanical properties of the membranes are presented in Fig. 4A. The curves had similar trends, by increasing strain, the curves diverged from the linear proportionality and afterward according to the α-TCP content and its distribution in PCL matrix, they exhibited significant changes on the mechanical properties such as strength and toughness. The mechanical properties of fibrous membranes confirmed significantly higher toughness and strength of α-TCP:PCL compared to PCL membrane (Fig. 4(B) and (C)). In details, the strength of PCL fibrous membrane reached to 2.92±0.31 MPa for PCL-1α membrane from 1.60±0.46 MPa for PCL, and by adding more amount of α-TCP nanopowder, the strength decreased significantly (1.89±0.54 MPa for 2α-PCL). Moreover, the incorporation of 1 wt% α-TCP nanopowder enhanced the toughness of the membranes by 1.57±0.10 and 3.11±0.72 fold for 0.5α-PCL and 1α-PCL, respectively, while tensile modulus did not significantly modulate. As demonstrate in Fig. 5, by adding more α-TCP up to 2wt.%, the mechanical properties get decreased due to the agglomeration of nanoparticles. So, 1α-TCP:PCL has the proper mechanical properties to use as GBR membrane application. However, the elongation of the membranes was not significantly different. Such similar results were demonstrated in other researches which demonstrated that by adding nanoparticles the mechanical properties improved [27-28]. To be precise, the incorporation of Diopside nanoparticles in PCL matrix increased the mechanical strength of scaffolds from 0.6±0.1 MPa (for PCL) to 4.9±0.1 MPa (for PCL with 3wt.% diopside) [21]. This increase in mechanical properties might be due to the energy-dissipating mechanism which occurs for incorporation nanoparticles in the polymer matrix [29].

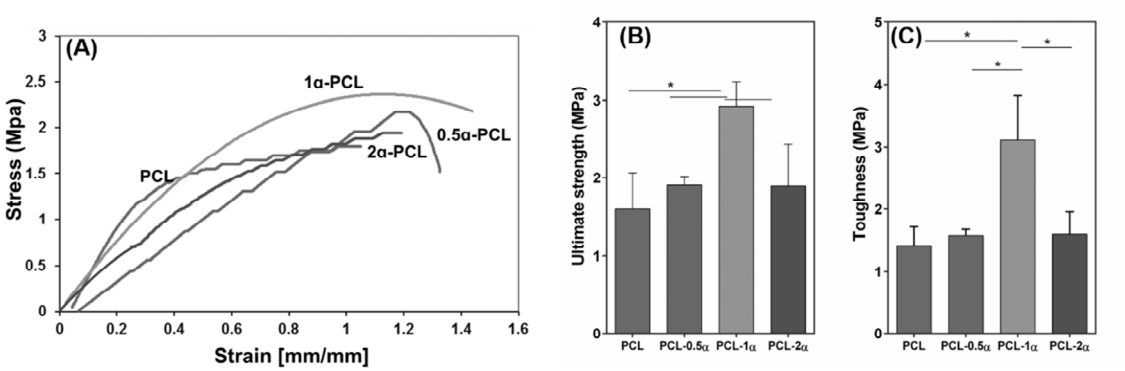

Fig.4. Mechanical properties of fibrous scaffolds (A) stress-strain curves (B) Ultimate strength and (C) Toughness of scaffolds.

4. Conclusions

In this study, we developed α-TCP:PCL fibrous membranes through electrospinning for bone tissue regeneration. The fibrous membranes exhibited slight decrease in pore sizes (34% smaller) in comparison to individual PCL membrane and the cellular migration into membrane was improve eventually. Results showed that 1α-TCP:PCL fibrous membranes exhibited significant improved structural properties in comparison to PCL membranes which yields in enhanced mechanical properties such as strength from 2.92±0.31 MPa (for 1α-PCL). In summary, our findings indicated that α-TCP:PCL fibrous membrane with the ability to address the issues of weak biocompatibility and small pore sizes of the electrospun synthetic membranes are potentially suitable constructs for guided bone regeneration.

References

[1] F. Yang, S. K. Both, X. Yang, X. F. Walboomers, J. A. Jansen, Acta biomaterialia 5, (2009), 3295-3304.

[2] J. Wang, L. Wang, Z. Zhou, H. Lai, P. Xu, L. Liao, J. Wei, Polymers 8, (2016), 115.

[3] J. Xue, M. He, Y. Liang, A. Crawford, P. Coates, D. Chen, R. Shi, L. Zhang, J. Mater. Chem. B 2, (2014), 6867-6877.

[4] H. Su, K. Y. Liu, A. Karydis, D. G. Abebe, C. Wu, K. M. Anderson, N. Ghadri, P. Adatrow, T. Fujiwara, J. D. Bumgardner, Biomedical materials 12, (2016), 015003.

[5] P. Carter, S. M. Rahman, N. Bhattarai, Journal of biomaterials science. Polymer edition 27, (2016), 692-708.

[6] S. D. McCullen, Y. Zhu, S. H. Bernacki, R. J. Narayan, B. Pourdeyhimi, R. E. Gorga, E. G. Loboa, Biomedical materials 4, (2009), 035002.

[7] J. H. Shim, J. B. Huh, J. Y. Park, Y. C. Jeon, S. S. Kang, J. Y. Kim, J. W. Rhie, D. W. Cho, Tissue engineering. Part A 19, (2013), 317-328.

[8] M. S. Kim, G. H. Kim, Materials Letters 120, (2014), 246-250.

[9] L.-n. Jia, X. Zhang, H.-y. Xu, F. Hua, X.-g. Hu, Q. Xie, W. Wang, J. Jia, Journal of Nanomaterials 2016, (2016), 1-10.

[10] K. Fujihara, M. Kotaki, S. Ramakrishna, Biomaterials 26, (2005), 4139-4147.

[11] G. Mestres, C. Le Van, M.-P. Ginebra, Acta Biomater. 8, (2012), 1169-1179.

[12] A. G. M. John P. Fisher, Joseph D. Bronzino, Donald R. Peterson, Tissue Engineering: Principles and Practices, 2012.

[13] M. Roozbahani, M. Alehosseini, M. Kharaziha, R. Emadi, Materials Science and Engineering: C, (2017).

[14] R. Enderle, F. Götz-Neunhoeffer, M. Göbbels, F. Müller, P. Greil, Biomaterials 26, (2005),3379–3384.

[15] RG. Carrodeguas, AH. De Aza, X. Turrillas, P. Pena, S. De Aza, J Am Ceram Soc 91, (2008), 1281–1286.

[16] RW. Nurse, JH. Welch, W. Gutt, J Chem Soc 220, (1959), 1077–1083.

[17] AM. Pietak, JW. Reid, MJ. Stott, M. Sayer, Biomaterials 28, (2007), 4023–4032.

[18] N. Golafshan, M. Kharaziha, M. Fathi, Carbon, (2016).

[19] M. Kharaziha, M. H. Fathi, H. Edris, Materials science & engineering. C, Materials for biological applications 33, (2013), 4512-4519.

[20] F. Causa, P. Netti, L. Ambrosio, G. Ciapetti, N. Baldini, S. Pagani, D. Martini, A. Giunti, Journal of Biomedical Materials Research, (2006)

[21] Y. Hosseini, R. Emadi, M. Kharaziha, A. Doostmohammadi, J. Appl. Polym. Sci. (2017)

[22] R. Aghdam, S. Najarian, S. Shakhesi, S. Khanlari, K. Shaabani, S. Sharifi, J. Appl. Polym. Sci. (2012)

[13] G. Mestres, C. Le Van, M.-P. Ginebra, Acta biomaterialia 8, (2012), 1169-1179;

[24] J. W. Reid, L. Tuck, M. Sayer, K. Fargo, J. A. Hendry, Biomaterials 27, (2006), 2916-2925.

[25] M. Kharaziha, M. H. Fathi, H. Edris, Composites Science and Technology 87, (2013), 182-188.

[26] N. Golafshan, R. Rezahasani, M. T. Esfahani, M. Kharaziha, S. Khorasani, Carbohydrate polymers, (2017).

[27] Y. Hosseini, R. Emadi, M. Kharaziha, Materials Chemistry and Physics, (2017);

[28] P. Wutticharoenmongkol, N. Sanchavanakit, P. Pavasant, P. Supaphol, Macromolecular bioscience 6, (2006), 70-77.

[29] A. Gaharwar, C. Rivera, C. Wu, G. Schmidt, Acta Biomater 7, (2011)