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The Application of Cell Sheet Engineering for Bone Tissue Engineering Purposes

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ABSTRACT

The use of scaffolds in combination with osteogenic cells has been the gold standard in Bone Tissue Engineering (TE) strategies. These strategies have, however, in many cases failed to produce the desired results due to issues such as the immunogenicity of the biomaterials used and cell necrosis at the bulk of the scaffold related to deficient oxygen and nutrients diffusion. We originally propose the use of cell sheet (CS) engineering as a possible way to overcome some of these obstacles. In a first stage we tested the potential of a single osteogenic CS to induce bone formation *in* vivo. Osteogenic CSs were fabricated by culturing rat bone marrow cells in thermo-responsive culture dishes. The CSs were recovered from the dishes using a low temperature treatment and then were implanted subcutaneously in nude mice. New bone formation was verified from day 7 post transplantation using x-ray, µ-CT and histology. It was also verified the presence of a vascularized marrow in the new formed bone after 6 weeks of transplantation supporting the conclusion that healthy bone tissue was formed after transplantation of the osteogenic CSs. In a second stage, we assessed the potential of adding endothelial cells to the osteogenic CS to improve the vascularization of the new formed bone. Human umbilical vein endothelial cells (HUVECs) were placed between two osteogenic CS and implanted for 1 week in nude mice. Histological evaluation of the recovered implants shows a higher degree of new mineralized tissue in the samples with HUVECs. Furthermore, in the same samples, perfused vessels positive for human CD31 marker were found meaning that the tansplanted HUVECs participated in the vascularization of the new tissue formed. These results therefore confirm the great potentiality of CS engineering to be used in bone tissue engineering applications.

INTRODUCTION

Currently, the gold-standard strategies to promote bone regeneration such as in critical bone defects, comprise the use of autologous bone grafts, allografts or materials like ceramics and metals (Jordan et al. 2004; Salgado et al. 2004; Dawson and Oreffo 2008). All of these strategies have drawbacks such as the availability of tissues, donor site morbidity and immunogenicity issues,, and deficient integration in the host tissue that limit their application range and their overall performance (Kneser et al. 2006; Dawson and Oreffo 2008). It has been accepted for a few years that new strategies are needed in order to address the challenges posed in this field. Tissue Engineering (TE)-based strategies have been trying to solve many of the referred problems. These approaches typically involve the use of different cell types suitable for bone TE, growth factors and 3D biodegradable scaffolds (Nerem and Sambanis 1995; Salgado et al. 2004). Such approaches, however, face in many cases serious problems such as the immune response to the implanted construct, inadequate biodegradability rate and the lack of vascularization which leads to cell necrosis in the bulk of the construct (Folkman and Hochberg 1973; Ishaug-Riley et al. 1998; Kneser et al. 1999; Holy et al. 2000; Pirraco et al. 2009).
Cell sheet (CS) engineering technique using poly(N-isopropylacrylamide) (PIPAAm) thermo-responsive dishes might constitute a useful alternative to solve some of the mentioned issues. This technique, as

proposed by Okano's group(Bittner et al. 1998; Yamato and Okano 2004; Yang et al. 2005). allows for the recovery of the cells within its own matrix to be used as intact single or multilayered CS to engineer transplantable tissues

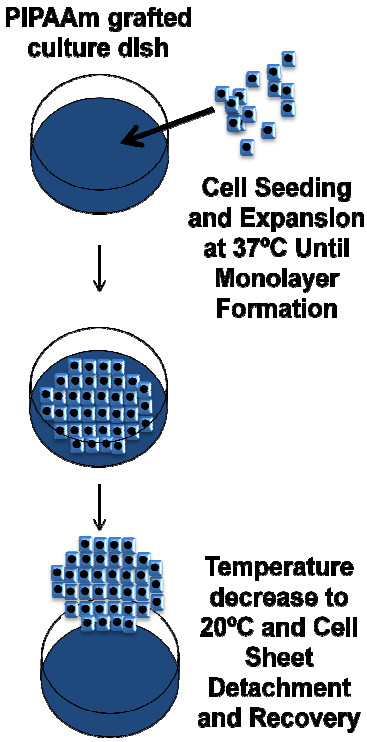

Figure 1 – Cell Sheet Production Using PIPAAm-Grafted Culture Dishes

So far this technology was proposed for the treatment of several tissues such as cornea (Nishida et al. 2004), myocardium (Shimizu et al. 2003), periodontal ligament (Hasegawa et al. 2005) and bladder (Shiroyanagi et al. 2003) but never for bone. The particular mechanical and biological properties of bone tissue make the application of CS engineering into bone rather complicated. Others have previously attempted to produce CS for the regeneration of bone tissue (Zhou et al. 2007; Akahane et al. 2008; Gao et al. 2009). Zhou and colleagues (Zhou et al. 2007) wrapped osteogenic CS made form porcine bone marrow stromal cells around polycaprolactone–calcium phosphate scaffolds. The post-subcutaneous implantation analysis of the construct showed some degree of new bone formation but mainly at the periphery of the scaffolds. The same pattern of new calcified tissue, around the scaffold, was achieved by Gao and co-workers (Gao et al. 2009), using a coral scaffold, and Akahane and colleagues (Akahane et al. 2008), using an hydroxyapatite ceramic scaffold. In the latter case, the CS were also ectopically implanted without any scaffold (Akahane et al. 2008) and new bone formation, albeit disorganized, was verified. In all of the three proposed approaches, new bone tissue was fairly disorganized, poorly vascularised and limited to the surface of the scaffolds around which the CS were wrapped. In contrast with the above referred works, where cells were detached using a cell scraper to obtain the CS, the use of thermo-responsive dishes allows for the use of an intact cell-cell and cell-matrix architecture, due to the well developed culture dish recovery method where the temperature is decreased to 20ºC provoking the hydration of PIPAAm and consequent loss of cell adhesion (Yamato and Okano 2004; Yang et al. 2005) (Fig 1).

In this work, we aimed, in a first stage, at studying the *in vivo* bone formation potential of osteogenic CS non-invasivelyrecovered by temperature decrease and, in a second stage, at combining osteogenic CS with HUVECs in order to promote the vascularization of the new formed tissue. Osteogenic CS were developed *in vitro* from rat bone marrow stromal cells, cultured in thermo-responsive dishes, and then characterized using histology anf immunohistochemistry. The developed sheets were subsequently transplanted subcutaneously to the dorsal flap of nude mice, at first one CS at a time and then stacking two CS with HUVECs between them. Implants were recovered at different time points post-transplantation and characterized using histology, immunohistochemistry and µ-CT for the detection of mineralization.

MATERIALS AND METHODS

Temperature-responsive culture surfaces

Thermo-responsive dishes (CellSeed, Tokyo, Japan) were prepared as previously described (Hirose et al. 2000). Briefly, N-isopropylacrylamide monomer in 2-propanol solution was spread onto 35 mm diameter culture dishes (BD Biosciences, Franklin Lakes, NJ). Dishes were then irradiated by electron beam, resulting in both polymerization and covalent grafting of the poly(N-isopropylacrylamide) (PIPAAm) onto the cell culture surfaces. PIPAAm-grafted dishes were rinsed with cold-distilled water to remove ungrafted monomer, and dried in nitrogen gas. Dishes were finally sterilized with ethylene oxide gas prior to experimental use.

Cell sheets fabrication

Bone marrow was flushed from the femurs of 4 weeks old male Wistar rats (Charles River, Yokohama, Japan). After vigorous pipetting to disaggregate any clumps, the suspension was placed over Histopaque 1083 (Sigma-Aldrich, Tokyo, Japan) and centrifuged at 2500RPM for 25 minutes. The mononuclear cell fraction was recovered after centrifugation and washed in phosphate buffered saline (PBS, (Sigma-Aldrich, Toyko, Japan)) to remove any remaining Histopaque. Cells were then seeded in 100 mm of diameter tissue culture polystyrene dishes and cultured in basal medium (DMEM (low glucose; Wako Pure Chemical Industries, Tokyo, Japan), supplemented with 10% fetal bovine serum (Japan Bioserum Co.Ltd, Hiroshima, Japan) and 100 units/ mL of penicillin–streptomycin (Sigma-Aldrich Japan, Tokyo, Japan)) at 37 ºC and in a 5% of CO2

humidified atmosphere. After 24 hours of culture, non-adherent cells were removed from the culture and the adherent cells (rat bone marrow stromal cells –rBMSCs) were then cultured until semi-confluence was achieved. Cells were detached using a 0.25% trypsin-EDTA solution (Gibco BRL LifeTechnologies, Carlsbad, USA) and seeded in 35 mm of diameter thermo-responsive dishes at a concentration of 2.5 x 10^5 cells per dish. Cultures were maintained for three weeks in osteogenic medium (basal medium supplemented with 10-8 M dexamethasone (Sigma-Aldrich, Toyko, Japan), 50 µg/mL ascorbic acid (Sigma-Aldrich, Toyko, Japan) and 10 mM beta-glycerophosphate (Sigma-Aldrich, Tokyo, Japan).

HUVECs and rBMSCs co-cultures

HUVECs at passage 3 were seeded, 3 days before the rBMSCs in thermoresponsive-dishes completed 3 weeks of culture, in half of the dishes at a density of 1 x 10^5 cells per dish. Co-cultures were maintained in ECGM (Lonza, USA) supplemented with 10^{-8} M dexamethasone, 50 µg/mL ascorbic acid (Sigma-Aldrich, Toyko, Japan) and 10 mM beta-glycerophosphate (Sigma-Aldrich, Toyko, Japan) for 3 days until transplantation.

Recovery of cells from thermoresponsive dishes

In order to recover the cells from the thermoresponsive dishes, culture medium was removed from the culture dishes and replaced with 1 mL of PBS. A poly(vinylidene difluoride) (PVDF) membrane(Immobilon-P, DURAPORE®, Millipore Corporation, Billerica, USA) with 2 cm of diameter was placed over the cells in the thermoresponsive dishes and incubated for 10 minutes at 20 ºC. After this time, CS spontaneously detached from thermoresponsive dishes. Some of the recovered CS were fixed in 10% formalin (Wako Pure Chemicals, Osaka, Japan) for posterior characterization. To produce the stacked CS, recovered osteogenic CS were placed over the co-cultures. Like for the single CS, this construct was incubated for 10 minutes at 20ºC and the stacked CS spontaneously detached from thermoresponsive dishes.

In vivo transplantation

The transplantation of the CS was carried out as previously reported (Obokata et al. 2008). Briefly, 6 weeks old male nude mice (Charles River Japan, Yokohama, Japan) (6 animals per transplantation time) were anesthetized with a constant flux of 4% of isofluorane. Dorsal skin was cut opened using 3x3cm cutting sides. Recovered CS were placed on mouse subcutaneous dorsal flap and left to adhere to the connective tissue of dorsal skin for 5 minutes. After that time, the PVDF membranes were removed and silicone membranes were placed over the CS to prevent the contact between the CS and the muscular tissue. Control mice (3 animals per transplantation time) were also prepared by implanting only silicone membranes in the case of single CS implantation, or two CS without HUVECs in the case of stacked CS implantation. Skin incisions were closed using 5-0 nylon sutures. Animals were kept with food and water *ad libitum*. After 7 days (single and stacked CS) and 3 weeks and 6 weeks (for single CS) of implantation, animals were euthanized with CO_2 and implants were recovered for characterization.

Histological characterization

After fixation with formalin, both in vitro recovered CS and implanted samples were embedded in paraffin, without demineralization, and 5 µm thick sections were made. Hematoxylin and eosin (H&E) staining was performed following standard protocols.

To assess mineral deposition Alizarin Red staining was performed. Briefly, a solution of 0.1 % of alizarin red (Sigma-Aldrich, Tokyo, Japan) was made in ddH2O and the pH was adjusted to 4.6. Sections were deparaffinized and 1 mL of alizarin red solution was added to each slide. Sections were observed in the microscope until correct amount of colour developed. Pink/purple colour was considered positive for mineral deposition. Sections were then washed in ethanol and xylene. Micrographs of the sections were taken after H&E and Alizarin Red stainings.

The identification of specific markers was carried by immunohistochemistry. Briefly, both CS and implant sections were incubated with a primary antibody (SRY(1/100), collagen I (1/200), osteocalcin (1/200), osteopontin (1/100), osterix (1/100) and CD31 (ready-to-use)) overnight, at 4 ºC and then for one hour, room temperature, with a biotynilated secondary antibody (DakoCytomation, Glostrup, Denmark). Sections were incubated with Streptavidin-HRP (DakoCytomation, Glostrup, Denmark) solution for 20 minutes and then treated with DAB chromogenic substrate solution (DakoCytomation, Glostrup, Denmark).

Stained sections were analysed with a Eclipse E800 microscope (Nikon, Tokyo, Japan).

Micro-computed Tomography

To investigate the 3D structure of the mineralized tissue formed after transplantation of single CS, non-destructive techniques, X-Ray and Micro-Computed Tomography (SkyScan, Kontich, Belgium) were used. Recovered implants, after paraffin embedding, were cut in half and scanned in a high-resolution mode of 11,32 µm x/y/z and an exposure time of 1900ms. The energy of the scanner used was 50 keV with 171 mA current. The µ-CT scans were followed by a 3D reconstruction of serial images.

RESULTS

Osteogenic Cell Sheets

The used CS engineering methodology allowed fabricating and recovering intact rat bone marrow-derived CS composed by a dense collagenous matrix

where cells were embedded. By micrograph observations, the estimated thickness of the CS after recovery was of about 30 µm. Although this value does not reflect the real thickness of the sheet *in vitro* that contracted after being release from the dish, it corresponds to the thickness of the implanted CS. Significant mineral deposition in the cultured CS was observed as evidenced by the intensity of the Alizarin Red staining. Immunohistochemistry against collagen I, osteopontin and osterix validated the osteogenic nature of the culturedCS. The results showed that the cultured CS were highly expressing these proteins, demonstrating their osteogenic character (Fig 2).

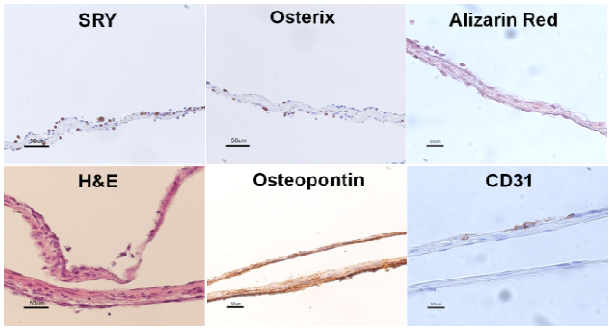

Figure 2 – Cell Sheets Characaterization Prior To Implantation

Implant characterization

Single CS implantation

In the case of single CS implantation, evidences of *in vivo* new bone formation after transplantation were confirmed by X-ray and µ-CT analysis. After 7 days of transplantation the amount of dense tissue is already significant and a notorious increase in the density of the neo-bone was observed as the transplantation time increased. (Fig 3)

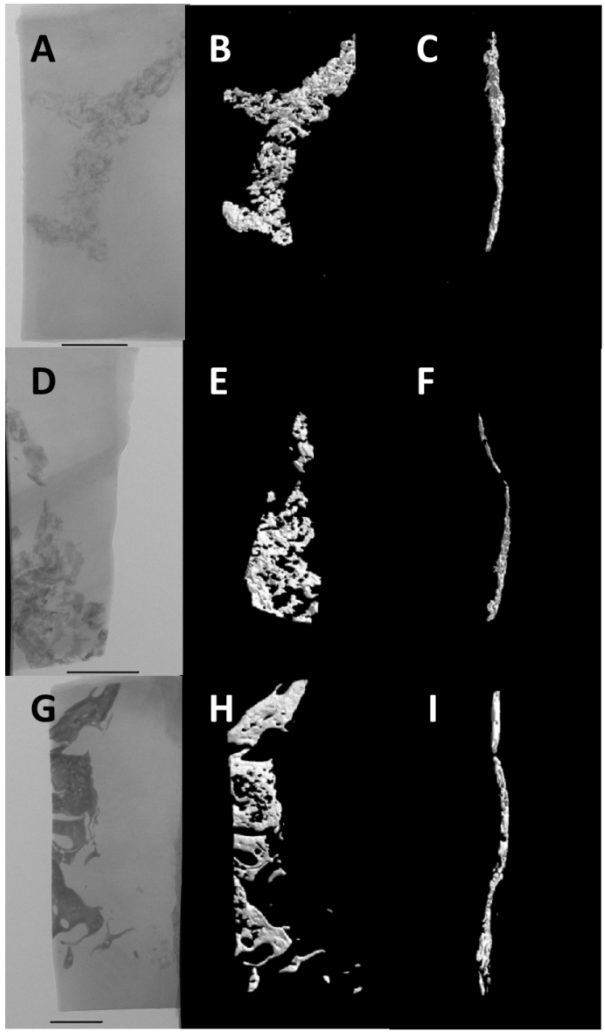

Figure 3 – Representative X-ray (A,D and G) And µ-CT Images (side [C,F and I] And Front View [B,E and H]) Of The Transplants (each divided in half after recovery) After (A-C) 7 Days, (D-F) 3 Weeks And (G-I) 6 weeks Of Implantation. Bars Represent 2 mm And Are Valid For All The Images.

These results were further confirmed by Alizarin Red/H&E staining (figure 4). Mineralized tissue was observed at the implant site just seven days post-transplantation. The amount of new-mineralized bone increased through-out the time of transplantation until it reached a maximum, 6 weeks post-transplantation.

Immunohistochemistry for osteocalcin showed that this protein was expressed at different locations even after only 7 days of transplantation. These positive sites seem to correlate to sites where new bone was being formed. With the increase of new bone formed with the transplantation time, it was clear that the majority of the cells that were positive for this protein were concentrated around the new tissue. This is an expectable outcome because this protein is directly related to matrix mineralization and, consequently to new bone formation (Boston 1992; Stein and Lian 1993; Muraglia et al. 2000).

The most striking feature observed in the new-bone at 6 weeks post-transplantation was the void largest patches

(figures 4D and 4E). This fact may indicate that bone remodelling was occurring, following the normal bone tissue metabolism where bone is constantly resorbed and formed (Frost 1986; Jee and Frost 1992; Robling et al. 2006). In fact figure 4F clearly shows osteoid deposition. Moreover, numerous cells could be found in those void marrow spaces, among which red blood cells, which seems to indicate vascularisation of the new formed bone. This fact reinforces the hypothesis that the new bone formed is being remodelled, since it the existence of a direct connection between vasculature and bone remodelling was previously proven (Streeten and Brandi 1990; Collin-Osdoby 1994; Parfitt 1994; Kanczler and Oreffo 2008; Parfitt 2008). Also very important to notice was the presence of osteocytes, as it is visible in figure 4E since, besides being the most common cells in bone tissue, these cells are believed to be regulators of bone homeostasis (Palumbo et al. 1990; Nijweide et al. 1996; Kamioka et al. 2001).

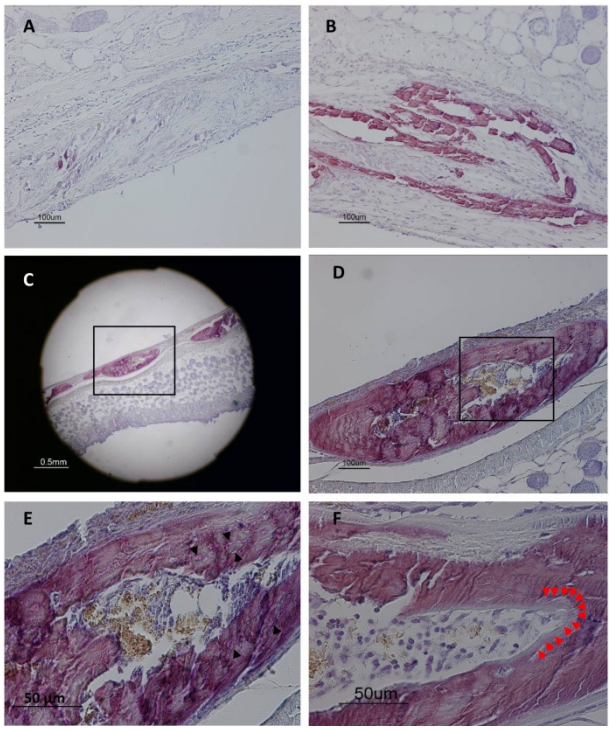

Figure 4 – Alizarin Red Staining For Mineralization For Implanted Single Cell Sheets Recovered After (A) 7 Days, (B) 3 Weeks And (C, D, E and F) 6 Weeks Of Implantation. Black Arrows Mark Osteocytes and Red Arrows Mark Osteoid.

Stacked CS plus HUVECs implantation

The H&E and Alizarin Red staining (Fig 5) perrmited to qualitatively compared of experimental (stacked CS plus HUVEC) and the control (stacked CS) conditions. The mineralized area in the experimental conditions is apparently larger than the one in the control conditions.

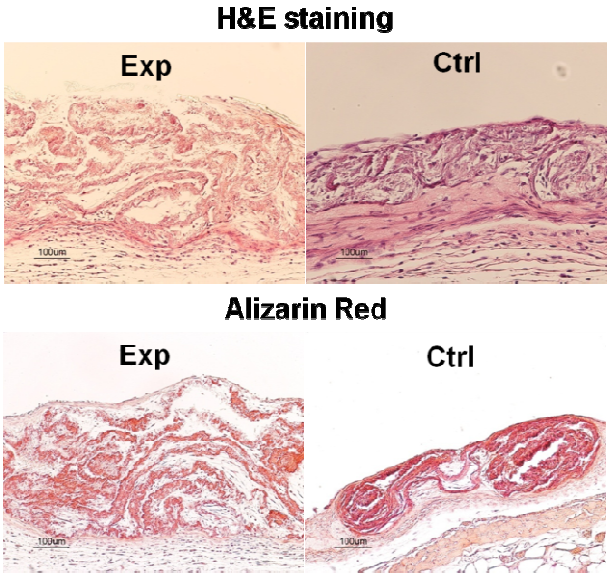

Figure 5: H&E and Alizarin Red Staining 1 Week After Implantation Of Stacked Cell Sheets. “Exp” Corresponds To The Stacked CSs With HUVECs. “Ctrl” Are The CSs Without HUVECs.

SRY protein staining allowed ascertaining on the distribution of the implanted cells *in vivo*. The CS were composed of male rat cells and implanted in female mice. SRY is a protein linked to male sex determination, therefore only present in males (Wallis et al. 2008). This way this protein was used to distinguish the CS cells from the host cells. It was possible to see that only a part of the cells in the mineralized area are positive (Fig 6), which means that host cells have been recruited to this area. This was true for both control and experimental conditions.

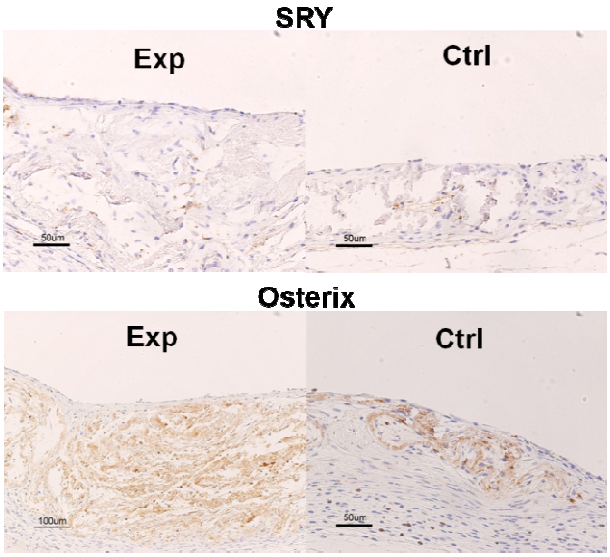

Figure 6: SRY and Osterix Immunostaining 1 Week After Implantation Of Stacked CS. “Exp” Corresponds To The Stacked CSs With HUVECs. “Ctrl” Are The CSs Without HUVECs.

The results for the osteogenic markers osterix (Fig 6) and osteopontin (Fig 7) confirmed the strong osteogenic character of the mineralized areas. Specifically looking at the osterix results and comparing with the results for SRY it may be concluded that more cells are positive for the former than for the latter. This means that the cells recruited from the host are committed to an osteogenic phenotype. These results seem to indicate that the implanted CS constructs induced the recruitment and osteogenic differentiation of host cells.

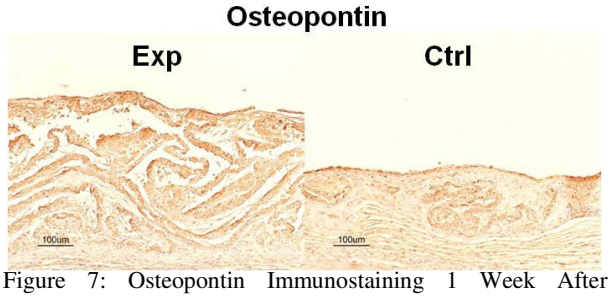

Figure 7: Osteopontin Immunostaining 1 Week After Implantation Of Stacked CS. "Exp" Corresponds To The Stacked CSs With HUVECs. "Ctrl" Are The CSs Without HUVECs.

To verify if HUVECs contributed to vessel formation *in vivo*, sections were stained for CD31 marker. Vessel-like structures were found throughout the sections, some of them perfused, confirming that the co-cultured HUVECs were capable of forming these structures *in vivo* (Fig 8). The ability of the implanted co-cultured CS to form vessels can explain why, in these conditions, the mineralized areas are larger than in control conditions.

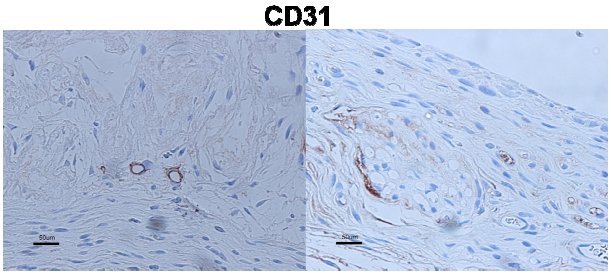

Figure 8: Immunostaining For Human CD31 Showing Blood Vessels Formed By HUVECs (brown)

Cell sheets have been previously used to create new bone tissue *in vivo* (Zhou et al. 2007; Akahane et al. 2008; Gao et al. 2009). The resulting bone tissue was however not satisfactory for TE applications since it was in most cases either disorganized (Akahane et al. 2008) or incapable of growing in the interior of the scaffold (Zhou et al. 2007; Gao et al. 2009). The common drawbacks of the use of scaffolds namely cell migration to the interior of the scaffold and oxygen and nutrient diffusion limitations (Folkman and Hochberg 1973; Ishaug-Riley et al. 1998; Kneser et al. 1999; Holy et al. 2000), resulted in poor outcomes. Nevertheless, Akahane and co-workers (Akahane et al. 2008) have implanted CS, recovered using a cell scraper, without any scaffold and new bone tissue was formed. Although cell-to-cell junctions were preserved using this method, the use of thermo-responsive dishes guarantees that both cell-matrix junctions and the ECM itself are preserved (Yamato and Okano 2004; Yang et al. 2005). The ECM can then serve as a natural glue that enable these sheets to be applied virtually in any anatomic site when implanted. Maeda and colleagues (Maeda et al. 2009) recently developed a device that allows a minimally invasive endoscopic transplantation of CS fabricated in thermo-responsive dishes. The expected developments of CS Engineering can open the door to deliver CS as *de facto* sheets for many applications using a minimally invasive surgery, in opposition to the hypothesis of just injecting them, as suggested by others (Akahane et al. 2008). The herein proposed methodology might be applied in flat bones defects, which, due to the nature of the CS, is the most obvious choice. However, stacking several CS in combination with endothelial cells, as we have also demonstrated, resulted in increased bone formation rate. Furthermore, the HUVECs used contributed to new vessel formation in the implants meaning that the increasingly important vascularisation issue (Pirraco et al. 2009; Sasagawa et al. 2009) was successfully addressed.

CONCLUSIONS

As demonstrated for other tissues, CS engineering is also a very promising technique for bone regeneration applications. This work demonstrated that new bone tissue, with very interesting characteristics, namely the presence of osteocytes, vascularisation and bone marrow formation, was formed from a single osteogenic CS. The stacking of osteogenic CS and the addition of HUVECs promoted new vessel formation and increased the rate of new bone formed. This way it is proven that CS Engineering is a technique with enormous potential in the bone TE field.

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AUTHORS BIOGRAPHIES

ROGÉRIO P. PIRRACO (rpirraco@dep.luminho.pt) was born in 1982 in Oporto, Portugal. At the present he lives in Oporto and works as a PhD student in the 3B's Research Group (Biomaterials, Biodegradables and Biomimetics), located in Guimarães. This is a research unit of Excellence integrated in the University of Minho and directly funded by the Portuguese Foundation for Science and Technology (FCT). Regarding his education background, in 2005 Rogério has concluded his four years graduation in Applied Biology at the University of Minho, Portugal. Rogério has started his contact with research during the last year of his graduation, when he developed his trainee period in the 3B's Research Group in the area of cell co-culture models for bone and cartilage Tissue Engineering. In 2006, he was admitted as a PhD student in the 3B's Research Group where he develops his work concerning co-cultures of cells for Tissue Engineering purposes, cell sheet engineering using thermally responsive surfaces and stem cell culture. During this time, he spent 12 months in Professor Teruo Okano's Institute of Advanced Biomedical Engineering and Science in Tokyo, Japan, where he acquired knowledge in cell sheet development and manipulation and its application in animal models. Rogério Pirraco was also a part of the organizing committee of the European chapter meeting of TERMIS-EU 2008, held in Oporto, Portugal. As a result of his work, Rogério has attended the most important international meetings in the Tissue Engineering field both with posters and oral communications. In 2007 European chapter of TERMIS, held in London, he received the prize for "Outstanding Student Contribution". He has published in refereed journals, in a book and in international conference proceedings. He is currently the chair of the Student and Young Investigator Section of the European chapter of the Tissue Engineering and Regenerative Medicine International Society

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ALEXANDRA P. MARQUES is an assistant researcher in the 3B's Research Group (Biomaterials, Biodegradables and Biomimetics) at the University of Minho, Portugal. In 1997 Alexandra P. Marques has concluded her four years graduation in Biochemistry, in the Faculty of Sciences of the University of Porto. During 1998 and 1999 she attended a one year specialisation course as part of the Biomedical Engineering Master/Doctoral Programme at the Faculty of Engineering of the University of Porto. In 2004 Alexandra P. Marques obtained her PhD on Materials Science and Technology – Biomaterials in the University of Minho in Portugal. Her PhD work was also carried out in cooperation with the University of Liverpool in UK, where she has worked for 18 months. From 2004 until 2006 she was Post-Doctoral Research Fellow at 3B's Research Group.

Her research focuses on the development of functional stem cells-based tissue engineering constructs for bone and skin applications. The design of innovative strategies for directing stem cell differentiation into the endothelial and epidermal lineages is of particular interest. New approaches for overcoming the vascularization hurdle currently impairing further advances in the regeneration of more than few millimetres in volume bone and dermal tissue engineering constructs are also of major interest.

Alexandra P. Marques has been involved in the co-supervision of final year projects of under-graduated students, Integrated Master and PhD projects. She is author or co-author of 26 papers published in referred journals, 6 book chapters and more than one hundred communications in major conferences of the field. She is also co-editor of the book *Handbook of Natural-based Polymers for Biomedical Applications.* She participated in the organisation of some conferences, is member of several scientific societies and acts as referee of numerous scientific journals. She is also of Assistant Editor of *Journal of Tissue Engineering and Regenerative Medicine*.

MASAYUKI YAMATO is a Professor of the Institute of Advanced Biomedical Engineering and Scienc Medical University. He was originally trained with a background in cell biology and biochemis decade, his research interests have been focused on the regeneration of various tissues and organs using cell sheets, instead of traditional tissue engineering approaches using cells

seeded into biod In particular, his work with both corneal and oral mucosal epithelial cell sheets has already been patients suffering from ocular surface dysfunctions. Presently, he is therefore currently engagec collaborations with physicians and surgeons from various medical departments, such as ophthaln gastroenterology, urology, and thoracic surgery; with the aim of taking regenerative medicine us level of basic laboratory science to clinical applications.
Research Interests: Tissue Engineering, Regenerative Medicine, Nano-biotechnology, Stem Cell
Awards:
The Award for Original Investigation, Japanese Society for Artificial Organs (1998)
The Award for Outstanding Paper, Japanese Society for Artificial Organs (2000)
The Award for Young Researcher, Japanese Society for Biomaterials (2002)
Young Investigator Award, Society for Biomaterials (2003)
Good Design Award, Japan Industrial Design Promotion Organization (2003)
Prizes for Science and Technology, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology Japan (2010)
The Yamazaki-Teiichi Prize, Yamazaki Foundation for Promotion of Material Science and Technology of Japan (2010)

TERUO OKANO is currently the Professor and Director of the Institute of Advanced Biomedical Engineering and Science (ABMES) at Tokyo Women's Medical University (TWMU) in Tokyo, Japan Currently, he is also an Adjunct Professor at the Department of Pharmaceutics and Center for Controlled Chemical Delivery at the University of Utah since 1994 as well as a Visiting Professor of Consolidate Research Institute for Advanced Science and Medical Care at Waseda University since 2004. He has been a Fellow of the American Institute of Medical and Biological Engineering since 1997 and also a Fellow of the International Union of Societies for Biomaterials Science and Engineering since 2000.
Prof. Okano's research interests currently involve the use of intelligent biomaterials for biomedical research. His research group has succeeded in harvesting cultured cells as viable and confluent cell layers by modifying temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm) onto ordinary polystyrene tissue culture dish surfaces proposing a new concept of "Cell Sheet Engineering". Prof. Okano is the author or co-author of more than 500 peer-reviewed journal articles as well as over 250
books and book chapters. He currently serves as an Associate Editor for a number of journals, including Nature, Nature Medicine, I of Biomedical Materials Research and Biomaterials. He received several awards such as the Outstanding Paper Award (1990, 1995, and 1996), given by the Controlled Release Society, and the Award of the Japanese Society for Biomaterials in 1992; the Outstanding Pharmaceutical Paper Award (1997) from the Controlled Release Society and the Clemson Award for Basic Research (1997) given by the Society for Biomaterials (USA), the Award of the Society Polymer Science, Japan (1998), the Founders Award (2000) from the Controlled Release Society, Leona Esaki prize
(2005) and Nagai Innovation Award from Controlled Release Society (2006). The latest additions are the
Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (2009), the Emperor's Medal with Purple Ribbon (National Meritorious Achievement Award) (2009), and the Yamazaki-Teiichi Prize (2009).

RUI L. REIS is the Director of the 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, a Research Unit of excellence based in U. Minho, Portugal. This is one of the most relevant groups in Europe on the field of Tissue Engineering and Regenerative Medicine. His group is one of the most active and interdisciplinary in the field, going from work with stem cells and its differentiation and expansion up to the in-vitro and in-vivo assessment of the functionality of the developed constructs. He has well established cooperation work with major research groups and companies all over the world. He has been the co-coordinator of four major EU research project, funded under FP6 of the European Commission. One of the main projects was the STREP "HIPPOCRATES" that had a 3 MEuros budget. He also coordinates the only European Network of Excellence (NoE) on Tissue Engineering, "EXPERTISSUES". This, still ongoing, highly funded NoE (budget of around 7.3 MEuros) is composed by 22 partners, several being industrial, from 13 countries, and is leading the way in all Tissue Engineering research in Europe. He has also coordinated the Marie Curie Early Stage Training Multi-site project "ALEA JACT EST" (total budget of 2.6 MEuros). He also coordinated the Marie Curie Series of Conferences "InVENTS ", that had a budget of around 0.5 MEuros to prepare 6 cutting-edge research conferences (all in Portugal) and 3 practical training courses at the highest level on the respective research fields. He has also coordinated the large INTERREG Project PROTEUS, with a budget of 1.4 MEuros aimed to develop new materials for different applications based on marine resources from Northern Portugal and Galicia. He is presently also involved in the large scale FP7 project DISCREGENERATION and coordinates two new strategic FP7 funded projects, the project FIND & BIND and the project SPECIAL each one with a budget of around 3.6 MEuros, as well as a new cross-broader large project IBEROMARE with a budget of around 2 MEuros. He is also the main responsible for several other projects funded by Portuguese, European and American biomaterials and polymeric industries and

for a range of bi-lateral concerted actions. At the present is his the principal investigator (PI) of grants totalising around 25 MEuros of which 8 MEuros are U. Minho funding. As a result of these projects he is presently an advisor of 140 post-graduation researchers from 18 different nationalities. Prof. Rui L. Reis is the CEO of the new European Institute of Excellence on Tissue Engineering and Regenerative Medicine Research, with headquarters in Minho and branches in other 19 locations throughout Europe. Rui L. Reis has also been awarded several prestigious scientific prizes. He has edited several books and journal special issues, organized different meetings and symposiums, and is the Editorial Board of many different journals. He is the Editor in Chief of the Journal of Tissue Engineering and Regenerative Medicine, John Wiley & Sons. Rui L. Reis is an author of more than 320 papers on scientific journals, around 152 book chapters in books of international circulation and more than 1000 communications in conferences, including around 125 plenary or invited talks delivered worldwide.