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Compositional changes to synthetic biodegradable scaffolds modulate the influence of hydrostatic pressure on chondrogenesis of mesenchymal stem cells

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Abstract

Mechanical cues such as hydrostatic pressure (HP) are known to regulate mesenchymal stem cell (MSC) differentiation. The fate of such cells is also strongly influenced by their substrate. The objective of this study was to test how different modifications of polycaprolactone (PCL) scaffolds would influence the response of MSCs to HP. Porcine bone marrow derived MSCs were cultured on PCL, PCL-hyaluronic acid (HA) and PCL-Bioglass® (BG) scaffolds for 35 d and stimulated with a HP bioreactor (10 MPa; 1 Hz; 2 h d^{-1}). Scaffold composition was found to modulate the response to HP. MSCs seeded onto both PCL and BG scaffolds responded positively to the application of HP, with increases in cartilage extracellular matrix synthesis and a reduction in type I collagen accumulation. This positive effect was not observed on HA scaffolds. The results of this study demonstrate that changes to scaffold composition can have a notable effect on the response of MSCs to bioreactor culture conditions.

1. Introduction

Cartilage is an avascular tissue formed by chondrocytes isolated in lacunae among a matrix of collagen and proteoglycans [1]. This tissue shows a limited self-repair capacity when it is injured (partial and full thickness lesions) [2, 3]. Some therapeutic options, such as microfracture, include the injure of subchondral bone to allow the invasion of the cartilage defect by bone marrow mesenchymal stem cells (MSCs), techniques that reduce the iatrogenic pain from the surgical intervention compared to other techniques [4]. Tissue engineering points to use scaffolds, cells and signalling factors to obtain a therapeutic option. The cell source for cartilage engineering in clinics has been autologous mature chondrocytes, the technique known as autologous chondrocyte transplantation, ACI [1, 5] or MACI [5] (implanting chondrocytes alone or seeded into a scaffold) but this procedures require two surgical interventions [2] and chondrocytes expanded *in vitro* dedifferentiate [6]. On the other hand MSCs are relatively easy to obtain and are able to differentiate towards chondrogenic lineage [7–10]. Nevertheless, their use in cartilage TE is hampered by the fact that they usually fail to produce good quality hyaline repair tissue [11] and that they do not differentiate to a 'stable adult hyaline chondrocyte' phenotype showing markers of hypertrophy and calcification [12, 13]. There is much interest in increasing knowledge about the factors that induce MSCs chondrogenesis *in vitro* and *in vivo*, in particular the properties of the three dimensional scaffolding system and the mechanical stress transmission to the cells from the environment.

With respect to the role of cell–material interaction, the aim of this paper is to determine how cell–material interactions modulate the effect of mechanical stimulation on MSC chondrogenesis. In this work

we use polycaprolactone (PCL), a synthetic polymer, that has been used previously to develop three-dimensional substrates with good mechanical properties and an interconnected pore structure that supports tissue growth and cartilage repair *in vivo* [14, 15]. On the other hand, PCL presents the typical drawbacks associated with biodegradable polyesters such as the absence in its structure of peptide sequences the cells are able to recognize, so cell–material interaction must be mediated by the proteins adsorbed over the material surface [16] or its high hydrophobicity [17]. A common way to improve cell function on synthetic polymer scaffolds is to modify the surface to increase the hydrophilicity, change the topography, or coat the scaffold with natural polymers to enhance proliferation and differentiation [16]. In this work, we develop scaffolds that introduce either a hyaluronic acid coating on the scaffold pore walls or Bioglass® particles. Hyaluronic acid (HA) is a natural polymer present in cartilage [17, 18] that may promote adhesion through the CD44 receptor and may increase cell adhesion, proliferation and chondrogenesis [19–22]. Bioglass is a silicon glass with CaO, Na_2O_3 and P_2O_5 widely used in bone tissue engineering but rarely in cartilage tissue engineering [23]. Silicon is a trace element with a fundamental role in normal collagen and glycosaminoglycans (GAGs) synthesis [24].

Culture conditions such as mechanical stimulus, growth factors, hypoxia and co-culture conditions are known to regulate chondrogenesis [25, 26]. Mechanical loading influences MSC differentiation and can be applied *in vitro* through different ways such as uniaxial compression or hydrostatic pressure (HP) [27, 28]. In this work, physiological levels of cyclic HP were applied to the scaffolds containing MSCs in order to mimic the 'strain-free' stress experienced by cells in the nearly incompressible fluid inside the cartilage matrix during joint loading [29]. It has been shown that HP loading applied at physiological levels [29–31] induces the expression of chondrogenic markers such as sulphated glycosaminoglycan (GAGs) or collagen II in MSCs and chondrocytes [31–33], and reduces the expression of hypertrophic markers such as Col I, Col X and MMP-13 and calcification of engineered grafts [34–36]. Nevertheless, it has been demonstrated in the literature that MSC show different response to mechanical stimulus depending the substrate [36, 37].

In order to test the influence of cell–material interactions on the effectiveness of HP stimulation, we compared the performance of bare PCL scaffolds with scaffolds with two surface modifications. In this study, hyaluronic acid coated scaffolds are PCL scaffolds in which the pore walls were coated with a thin layer of HA that provide specific cues for interaction with MSCs but, on the other hand, hinders the adsorption of characteristic adhesion proteins onto the scaffold surfaces. On the other hand, BG inclusion in polymer matrix should increase hydrophilicity, roughness, and protein adsorption improving cell adhesion and migration [23, 38].

We cultured porcine bone marrow derived MSCs for 35 d on PCL, PCL-hyaluronic acid (HA) and PCL-Bioglass® (BG) scaffolds in a HP bioreactor. Cell phenotype and tissue *in vitro* formation was assessed through the study of the production of ECM (GAGs and Collagen), histological and immunohistological staining.

2. Materials and methods

2.1. PCL and BG scaffold fabrication

PCL and PCL + 5% BG scaffolds at 20% of polymer in dioxane were prepared as described previously [39] by a mixed particle leaching/phase separation process. Briefly, in the case of pure PCL scaffold, the PCL solution was mixed with PEMA particles in a relationship 1:1 and frozen in liquid nitrogen. Dioxane was removed with ethanol at −20 °C after change the ethanol three times and after porogen was removed with ethanol at 37 °C after 14 washes. PCL + 5% BG samples were produced with the same protocol but using a suspension of Bioglass microparticles in the PCL solution. Bioglass microparticles (MO Sci Corporation, 20 μm of size) at 5% W/W respect to PCL were dispersed in dioxane by ultrasound stirring. Once mineral particles were dispersed in the solvent PCL was added with continuous stirring for 16 h until obtaining a homogenous suspension. As a result, in PCL + 5%BG, the mineral particles are dispersed inside the polymer matrix. Cylindrical samples, 5 mm diameter and 3 mm thick were cut out of the sheets obtained as a result of the production process.

2.2. HA sample preparation

PCL scaffolds were infiltrated with a 2% (w/v) HA (Hyaluronic acid sodium salt from streptococcus equi sp., Sigma-aldrich) solution in 0.2M NaOH mixed with a 2:1 molar ratio of divinyl sulphone (DVS) (Sigma-Aldrich). The sample filled with HA solution were left over a dry membrane for 1 h at room temperature to allow the crosslink reaction between HA chains and dried at 37 °C for 48 h. As a result from this protocol, HA is expected to form a thin, low swelling coating on the scaffold pore walls. Scaffolds were washed repeatedly with water. The samples were sterilized with 70% ethanol and dried.

2.3. Cell harvesting and subculturing

Porcine MSCs were isolated from the femora bone marrow of one donor using a modified protocol for human MSC [40]. The cells were seeded at 4×10^5 cells cm^{-1} in a T75 cm^2 culture flask and amplified in monolayer culture until passage 1 with DMEM GlutaMAX (Gibco), enriched with 10% of FBS (Gibco), 2% penicillin/streptomycin (P/S) (Gibco) and 125 μg ml^{-1} amphotericin B (Sigma). Then and

up to passage 4 the cells were cultured in T175 cm^2 culture flasks seeded with 1.8×10^4 cells cm^{-2} in presence of DMEM GlutaMAX, enriched with 10% of FBS, 2% P/S and 5 ng ml^{-1} recombinant human fibroblast growth factor-2 (FGF-2) (Prospec Bio).

2.4. Cell culture

Prior to cell seeding samples were cleaned in PBS, sterilized in ethanol 70% and conditioned overnight in DMEM supplemented with 2% P/S and 10% FBS in as described previously [39]. Excess of culture medium was aspirated with a pipette and scaffolds were deposited in a 3% agarose mould to encapsulate the scaffold to avoid cells escape from the scaffold consequence of cell seeding protocol.

Cells from passage 4 were detached and diluted in order to obtain a final concentration of approximately 5×10^5 cells/scaffold. For PCL and PCL + BG scaffolds 20 μl of cell suspension were pipetted and for PCL-HA scaffolds cell suspension was injected inside each scaffold. Samples were incubated for 135 min in the incubator at 37 °C to allow for cell adhesion. After this period, all scaffolds were supplemented with DMEM GlutaMAX enriched with 10% FBS, 2% P/S, 1% sodium pyruvate (Sigma), 1.5 mg ml^{-1} bovine serum albumin (Sigma), 40 μg ml^{-1} l-proline (Sigma), 1X insulin-transferrin-selenium (Sigma), 10 ng ml^{-1} recombinant human transforming growth factor-β3 (TGF-β3) (R&D Systems), 40 μg ml^{-1} dexamethasone (Sigma), 50 μg ml^{-1} ascorbic acid (Sigma) and 4.7 μg ml^{-1} linoleic acid (Sigma). The samples were cultured at 37 °C in hypoxic conditions (5% CO_2–5% O_2). Culture medium was changed twice a week for 35 d and samples were collected at 1 and 35 d.

2.5. Application of HP

The HP loading was carried in a custom made bioreactor [33] sealing the samples (six samples from each group) at day 14 inside sterile plastic bags with 15 ml (2.5 ml by sample) of medium. Mechanical stimulus was started at 14 d to allow the cells synthesizing a pericellular matrix, since the presence of a mature pericellular matrix is a requirement for the cells to sense the mechanical stimulus [27, 41]. The HP loading protocol consisted in a dynamic pressure (max pressure 10 MPa) at a frequency of 1 Hz for a period of 2 h d^{-1} five times per week along 3 weeks.

2.6. Mechanical characterization

Equilibrium and dynamic elastic moduli were determined by a stress relaxation test were performed using a (Zwick Z005, Roell) machine with 5N load cell according previous work [41].

2.7. Biochemical analysis

Samples were removed at 1 and 35 d, four samples for each type of scaffold were analysed. Samples were washed with DPBS and stored at −80 °C until the analysis was performed. After samples thawing, cells were digested adding papain at 125 μg ml^{-1} (Sigma-Aldrich) during 18 h at 60 °C to solubilize the biomolecules and protect it from cell enzymes.

2.8. Total collagen content

The content of collagen was determined doing the hydroxyproline assay using the protocol described by Kafienah [42]. Quantities of hydroxyproline were determined from a calibration curve realized using hydroxyproline standard and the amount of collagen was calculated using a value of hydroxyproline-to-collagen ratio of 1:7.69 [43].

2.9. GAG content

The content of sulphated GAGs was determined using Blyscan assay kit (modified version of DMMB assay) (Biocolor). Quantities of sulphated glycosaminoglycan were determined from a calibration curve realized using chondroitin sulphate standard provided in the kit.

2.10. DNA content

The total DNA present in the samples was measured using DNA Quantitation Kit (Sigma) following the manufacturer's instructions. Quantities of DNA were determined from a calibration curve realized using DNA standards. To determine the seeding efficiency the amount of total DNA in the sample was divided by the amount of DNA present in a single porcine MSC calculated from known cell concentrations (9.55 pg/cell). The number of cells obtained was divided by the theoretical number of seeded cells (5×10^5 cells/sample).

2.11. Histological analysis

Samples for microscopy were washed in DPBS and fixed with 4% paraformaldehyde (PFA) at 4 °C for 24 h. Samples were washed twice with DPBS to remove the PFA and stored at 4 °C in DPBS until analysis were performed. Samples were first dehydrated through a series of increasing percentage alcohol (70%, 80%, 90% y 100%), after this was immersed in absolute ethanol/polyester wax 50:50 overnight and finally included in a mould with polyester wax (Electron Microscopy Sciences) and cured 48 h at room temperature. The embedded scaffolds were cut longitudinally using the microtome Leica RM2025 in 10 μm thick sections. Sections were stained either for GAG with 1% Alcian Blue (counterstaining for nuclei with 0.1% nuclear fast red) or for collagen with 0.1% Picro-Sirius Red (counterstaining of cells with Harris hematoxylin). The collagen inmunohistochemical staining was carried out following a modified Dako kit staining protocol (EnVision® + dual Link System-HRP, Dako-Cytomation), adding an antigen retrieval step after endogenous peroxidase activity inactivation. After endogenous peroxidase inactivation, samples were

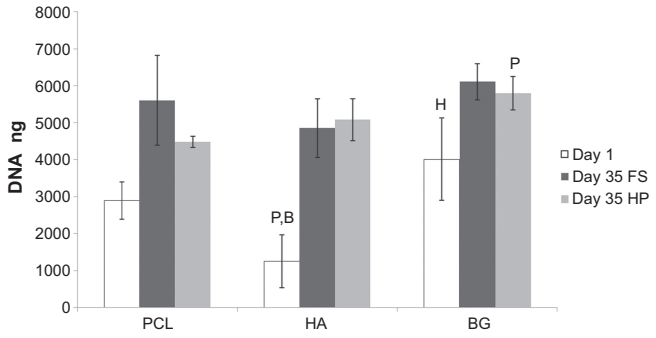

Figure 1. DNA content. Total DNA of porcine MSC after seeding at day 1 (white bars) and at day 35 under free swelling (dark grey bars) and hydrostatic pressure (soft grey bars) in PCL, BG and HA scaffolds. Results are averaged from $n = 4$ experiments. Significance ($p < 0.05$) compared to similar group is signalled as: (P) PCL, (B) BG and (H) HA samples for each group at the same time and culture condition.

incubated with pepsin (5 mg ml^{-1} in 5 mM HCl) 45 min at 37 °C. The samples were blocked and incubated 1 h in presence of anti-collagen I antibody (1:100) (Abcam) or anti-collagen II antibody (1:100) (ChemiconInc). After antibody incubation the samples were washed and incubated in presence of HRP-labelled polymer. Finally the sample was revealed adding the substrate-chromogen, dehydrated and mounted the sample with Entellan mouting media (Electron Microscopy Sciences).

2.12. Statistical analysis

Samples homogeneity was analysed doing a Levene's test to choose the correct statistical analysis. If Levene's test was positive a Student t-test or one factor ANOVA was chosen; but if it was negative a non-parametric test was used; differences were considered significant for $p < 0.05$. In all figures, error bars represent standard deviation.

3. Results

The total DNA content of the scaffolds at day 1 was used as a measure of the cell seeding efficiency of the scaffolds (figure 1). The cell seeding efficiency was significantly lower in the HA scaffolds (27.5% ± 15.66%), compared with PCL (63.56% ± 11.21%) and BG (88.08% ± 24.43%) scaffolds which were not significantly different from each other. DNA content increased with time for all three scaffold types, demonstrating they supported MSC proliferation (ratios of proliferation between 1.5 and 4). Proliferation was highest in the HA scaffolds, as the values at 35 d were similar to BG and PCL samples. HP did not significantly affect MSC proliferation within the scaffolds.

As seen from GAGs and collagen levels normalized to total DNA at day 35 (figure 2), MSCs cultured inside PCL and BG scaffolds responded positively to the application of HP, showing both significantly higher production of GAG and collagen than their respective FS control. On the contrary, in HA scaffolds loading had no significant effect on GAG production, and moreover it had a significant and negative impact on normalized collagen levels. On the other hand the collagen/GAGs ratio values obtained (PCL: FS = 2,12 ± 0.35 HP = 2,71 ± 0,33; HA: FS = 1,55 ± 0,04 HP = 2,03 ± 0,44; BG: FS = 1,46 ± 0,16 HP = 1.84 ± 0,41) showed that HP have a positive effect over collagen/GAGs ratio in PCL scaffolds with values more similar to 1.67 (the collagen and proteoglycans content in the native tissue [44]), suggesting a positive effect on MSC chondrogenic differentiation.

The Picro-Sirius Red and Alcian Blue staining shown in figure 3 shows the distribution of the cells and secreted extracellular matrix in the different MSC seeded scaffolds after 35 d in culture. In all the samples, the polymer matrix appears grey, whereas the pore space appears with the background colour (if void) or coloured if there is presence of cells or extracellular matrix. In free swelling conditions cells seems to aggregate into large clusters, mainly at or just under the surface of the scaffold. On the other hand loading seems to improve the distribution of cells and the MSCs cultured under HP, with small cell aggregates distributed throughout the scaffold. It is noteworthy that in some samples, the typical structure of chondrocytes isolated in their lacunae can be observed (black arrows in figure 3(A)).

As seen in figure 3(A), alcian blue staining, which stains negatively charged proteoglycans and glycoaminoglycans, showed a prominent staining in the centre of the samples whereas the edges were poorly stained. Relatively homogenous alcian blue staining was observed within HA scaffolds (scaffold colour changes from grey to blue-grey due to the staining of HA deposited as a very thin layer on the pore walls and inside the scaffold struts' microporosity), whereas PCL and BG show more localized staining. Collagen staining in all samples is more marked at the scaffold edge and around the cell aggregates (figure 3(B)) and collagen repartition inside the samples was improved

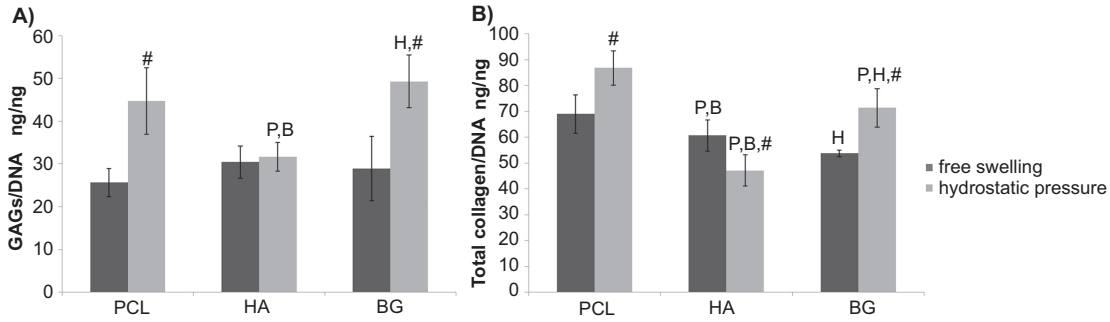

Figure 2. GAGs and collage content. GAGs (A) and collagen (B) levels normalized to total DNA at day 35 under free swelling (dark grey bars) and hydrostatic pressure (soft grey bars) in PCL, BG and HA scaffolds. Results are averaged from $n = 4$ experiments. Significance ($p < 0.05$) compared to similar group is signalled as: (P) PCL, (B) BG and (H) HA samples for each group at the same time and culture condition and (#) statically cultured sample from the same type at the same time.

under HP. The pores of the PCL scaffolds appeared to be homogenously filled with a collagenous matrix. On the other hand in HA scaffolds, most of the collagen is deposited on the outside of the construct, whereas BG situation is intermediary, with most collagen on the surface but with some deposition within the body of the construct.

Modulation of cell differentiation through the interplay of scaffolding material and culture conditions was also studied by examining collagen I and II deposition using immunohistochemical staining. Immunochemical staining for collagen I (COL I) presented in figure 4 follows the staining pattern for picro-sirius red. The macroscopic distribution was quite homogenous, only in HA samples were there areas without extracellular matrix observed, mainly in the bottom part of the scaffold. In the zones where staining was strong, a fibrillar collagen structure and organization of cells along the fibres were visible.

The inmunohistochemical staining for collagen II, presented in figure 5 follows the staining pattern for GAGs. The macroscopic distribution was not homogenous, with greater type II collagen deposition observed in the centre of the scaffolds and the upper surface as seen in the Alcian blue staining.

4. Discussion

Using synthetic substrates to modulate MSC response to culture conditions such as hydrostatic loading or hypoxia would be very useful for cartilage tissue engineering. Our group has previously worked with PCL scaffolds with a highly interconnected structure suitable for cell seeding and colonization, showing their utility for cartilage tissue engineering [14, 15, 45]. In order to verify the hypothesis that cell–material interaction may lead to a modulation of the response of MSCs to HP stimulation in chondrogenic medium, we used PCL scaffolds modified with hyaluronan or Bioglass®.

Scaffolds show a macroporous interconnected structure that allow for easy cell seeding and proliferation. Acellular scaffolds equilibrium moduli (PCL = 219,4 ± 58,8 kPa; HA = 326,2 ± 124,5 kPa and BG = 203,6 ± 70,3 kPa) were of a similar order of magnitude to values from the superficial region of articular cartilage from porcine joints [46]. The high hydrophobicity of PCL scaffold can represent a drawback to cell penetration and even distribution inside the scaffold; hyaluronic acid and Bioglass® were introduced inside the composites to lower the hydrophobicity and improve the biologic response. Bioactive glass particles inclusion in polymer may increase cell adhesion, but BG introduction only increases slightly the cell seeding efficiency compared with PCL samples as has been observed in previous works [39]. On the other hand, HA samples showed a significantly lower cell seeding efficiency compared to BG and PCL samples as already observed in our previous work [45], despite the use of agarose moulds to help retain cells within the scaffold during seeding. Lower cell seeding efficiency is thought to be due to the lower protein absorption over the hydrogel [16] and possibly due to the cell seeding method. Cell distribution was heterogeneous in all samples, but gained homogeneity when HP was applied. As HP has been previously shown to modulate cell migration [47], it is likely responsible for the more homogeneous distribution of both cells and ECM. Moreover cell growth was affected by the substrate used; whereas PCL and BG induced cell attachment to the pore wall, growth of cell aggregates was observed inside the pores of the HA scaffolds. On the other hand the synthesis of biomolecules was affected by the scaffold composition. The effect of the scaffolding material was evaluated by the Collagen/GAGs ratio. A value of 1.67, typical of that found in healthy cartilage, was used as [44]. BG had a positive effect on the Collagen/GAGs ratio compared to the unmodified PCL scaffold. BG is a bioactive glass whose main component is silicon, as a fundamental ion in cartilage and bone development as it plays a role in the regulation of collagen and GAGs synthesis [24].

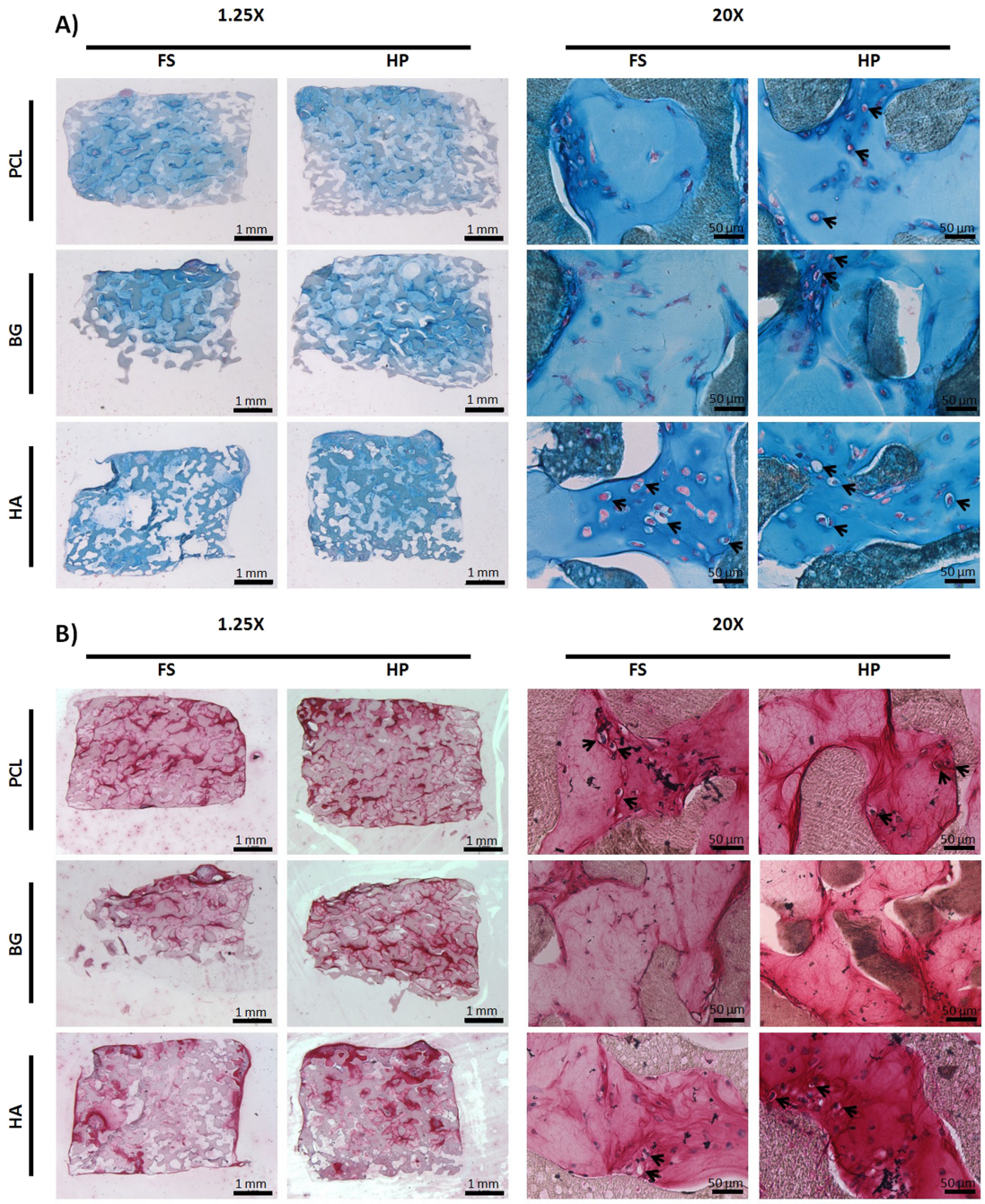

Figure 3. GAGs and collagen staining. (A) Picro-Sirius Red staining for collagen and (B) Alcian Blue staining for GAGs at day 35. (PCL: PCL samples; BG: PCL + 5% BG samples; HA: PCL-HA samples; FS—Free swelling samples; HP—hydrostatic pressure samples). (A): GAGs appear in blue and cells in pink. (B) Collagen appears in red and cells in purple. Black arrows points chondrocytes isolated in their lacunae (magnification = 1.25X and 10X. Scale bar = 1 and 100 μm).

On the other hand, HA samples did not show any improvement over collagen and GAGs content compared to bare PCL scaffolds, despite the fact that it is an important component of mature cartilage ECM. Differences in cell distribution, growth and localization correlated with ECM histological staining.HP generally favoured a more marked ECM deposition and led to a more homogeneous staining, without the intense staining at the edge observed generally in static cultures [39] HP loading is known to increase the expression of proteoglycans and collagen: [32] in our study, cells' ECM synthesis was significantly affected by the mechanical stimulation. Enhanced deposition of matrix was seen in histological cuts stained with either Picro-Sirius Red or Alcian Blue which matches with collagen I and collagen II pattern respectively. Sustained deposition of collagen was observed possibly due to the hypoxic conditions [48], as previous experiments with long cultures of MSC in normoxic conditions in such scaffolds showed poor results (data not published yet). However the presence of the collagen I as negative marker of hyaline cartilage shows

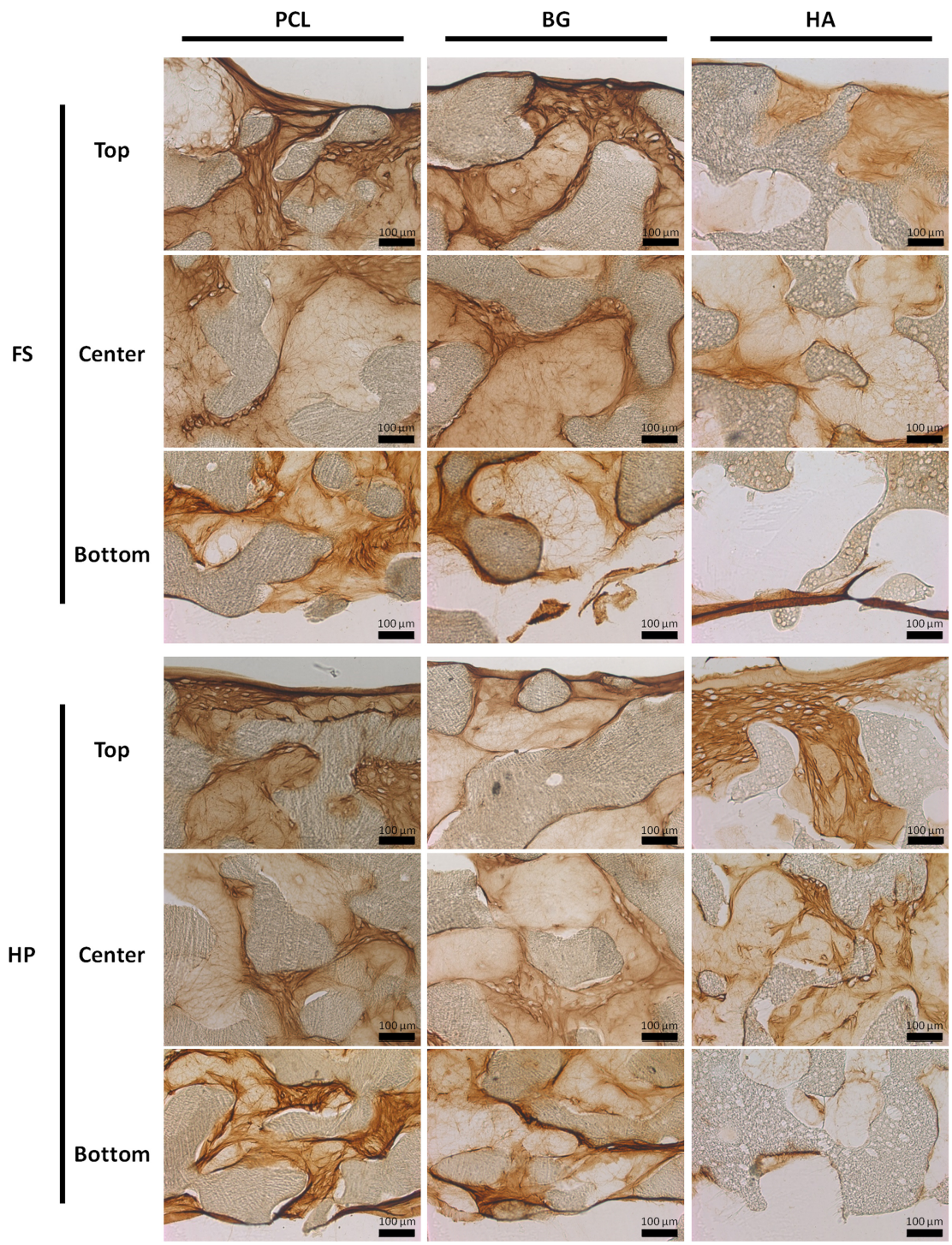

Figure 4. Collagen type I staining. Microscopic views of anti-collagen I inmunohistochemical staining of scaffolds cultured with bMSC cells at day 35. (PCL: PCL samples; BG: PCL + 5%BG samples; HA: PCL-HA samples). Scaffold appears grey and collagen is brown. (magnification = 10X. Scale bar = 100 μm).

the limitation of chondrogenic differentiation, but we cannot compare with collagen II because the staining intensity is significantly influenced by various factors.

On the other hand, most interestingly, the substrates modulated the cell response to HP, whereas all substrates led to similar quantitative ECM synthesis levels under free swelling conditions; only the PCL and BG scaffolds showed a positive response to loading. In contrast HP appeared to have little impact ECM accumulation with the HA scaffolds and over the Collagen/GAGs ratio. This is likely to be related to the mechanism and strength of the cell adhesion to the different substrates. Whereas cells interact with BG and PCL scaffolds through adsorbed proteins and integrin signalling, in the case of HA samples protein adsorption is greatly reduced and interaction may occur through CD44 binding. As observed in the results section, COL I deposition pattern was very

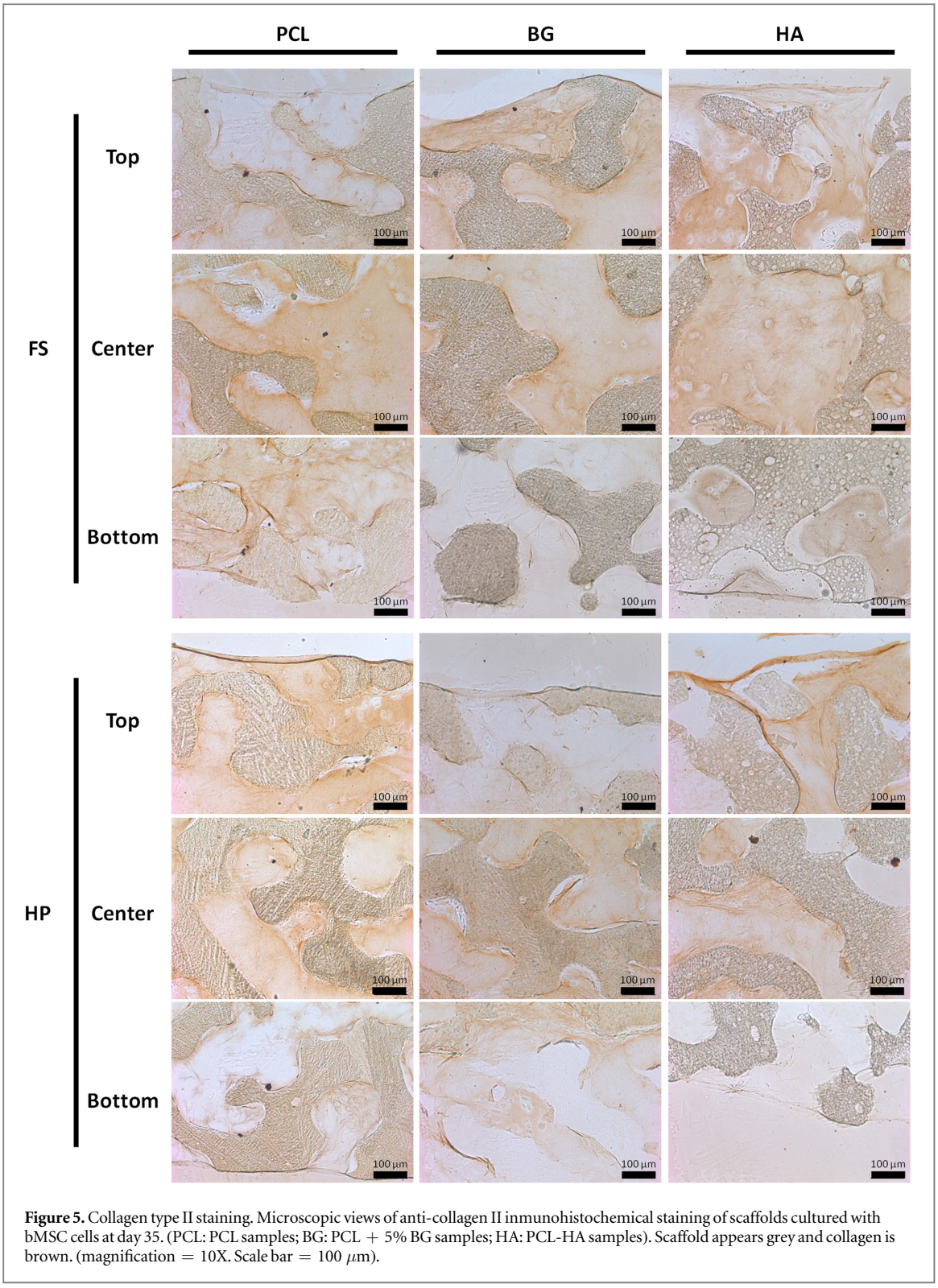

Figure 5. Collagen type II staining. Microscopic views of anti-collagen II inmunohistochemical staining of scaffolds cultured with bMSC cells at day 35. (PCL: PCL samples; BG: PCL + 5% BG samples; HA: PCL-HA samples). Scaffold appears grey and collagen is brown. (magnification = 10X. Scale bar = 100 μm).

different between adhesive (PCL and BG) and less adhesive (HA) samples. Strong fibres with intense stain, consistent with the application of significant cell contractility, were observed in PCL and BG samples in FS conditions, and lessened under HP, whereas this was not observed to the same extent in HA samples. In literature it is described that cell–matrix interactions through integrin binding has been shown to inhibit MSC chondrogenesis [36, 49], and whereas agarose gels (that promote cell rounding) were able to support chondrogenesis, fibrin gels (that induce cell spreading) showed less GAGs expression [36]. On the other hand, in the same study, only fibrin gels showed a positive response to HP whereas agarose samples did not; moreover mechanically induced enhancement of chondrogenesis in PEG hydrogels was shown to be integrin dependent [37]. Thus, different integrin implication in PCL and BG versus HA scaffolds could

explain why HP showed a positive effect on ECM production in PCL and BG scaffolds but not in HA scaffolds, in which HP had little or no effect, possibly because HA limits the integrin binding.

5. Conclusions

Culture of MSCs on synthetic rigid scaffolds can lead to positive results when cultured using chondrogenic factors such as TGF-β3, hypoxia and intermittent HP. Such scaffolds, once cultured, show modulus values in the range of normal cartilage tissue and should thus be biomechanically apt for implantation. Small composition changes in the scaffolding materials lead to different MSC response to intermittent HP. As a result, a typical response (increase of ECM production) is observed in bare PCL and Bioglass containing scaffolds. Distribution and morphology of the deposited ECM was also greatly changed by HA coating. Such changes are thought to be related with differential expression and use of cell surface receptors and their associated pathways.

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