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Injectable iron-modified apatitic bone cement intended for kyphoplasty: cytocompatibility study

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Abstract In this study, the cytocompatibility of human ephitelial (*HEp*-2) cells cultured on new injectable iron-modified calcium phosphate cements (IM-CPCs) has been investigated in terms of cell adhesion, cell proliferation, and morphology. Quantitative MTT-assay and scanning electron microscopy (SEM) showed that cell adhesion and viability were not affected with culturing time by iron concentration in a dose-dependent manner. SEM-cell morphology showed that *HEp*-2 cells, seeded on IM-CPCs, were able to adhere, spread, and attain normal morphology. These results showed that the new injectable IM-CPCs have cytocompatible features of interest to the intended kyphophasty application, for the treatment of osteoporotic vertebral compression fractures.

1 Introduction

In the last years, a large number of studies have been conducted to prove the biomedical applicability of iron-modified biomaterials in thermotherapy procedures [1–3], drug targeting [4], or as contrast agents in magnetic resonance imaging [5]. However, requirements of clearance of iron from the body often arise.

Iron is required from eukaryotic cells for survival and proliferation. The cellular iron metabolism is apparently sine qua non for cell replication; particularly, when cells are subjected to acute grow conditions [6]. The biological importance of iron is largely attributable to its chemical properties as a transition metal [7]. The most intracellular free iron is in the ferric state (Fe^{3+}) and during the intracellular events is reduced in a ferrous form (Fe^{2+}), which catalyzes free radical formation in the Fenton reaction [8]. However, in extreme case, an excess of "free" reactive iron might exceed the cell homeostatic capacity, thus compromising its integrity [6–8].

Calcium phosphate bone cements (CPBC) are clinically used in a wide range of applications, due to their ease of use, conformability, excellent plastic behavior before hardening, and high biocompatibility after being implanted in vivo [9]. Apatitic calcium phosphate cements set in situ by forming a net of entangled crystals of bone-like hydroxyapatite [10, 11] that has demonstrated to show good osteointegration [12, 13]. These characteristics make CPBCs promising materials for bone repair in dental and orthopaedic applications (to stabilize bone fractures, bone tumours, and osteoporosis) [14–17]. Despite all these advantages, CPBCs lack of mechanical strength and this limits their applications to non-load bone bearing situations [18]. Moreover, with the advent of minimally spinal invasive surgery techniques (vertebroplasty and kyphoplasty) it has been put forward that apatitic cements are difficult to inject into the compression fractured osteoporotic vertebras [18–20].

However, it has been showed recently that iron modification improves the mechanical strength and the

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injectability of apatitic bone cements [21, 22], which is of interest to spinal applications. Taking into account the comments given earlier, it is necessary to show at this stage that iron-modified calcium phosphate cements (IM-CPCs) are cytocompatible. For this reason, the cytocompatibility of the new IM-CPCs has been approached in this study by analyzing and quantifying the response (adhesion, morphology, viability) of human epithelial *HEp*-2 cells seeded on different IM-CPCs substrates.

2 Materials and methods

2.1 Cement substrates

Cement powder's phase was made of 70 wt% a-tricalcium phosphate (α-TCP; α-$Ca_3(PO_4)_2$ and 30 wt% of calcium sulphate dihydrate (CSD; $CaSO_4 \cdot 2H_2O$; Sigma-C3771) used as a porosity control agent to improve further in vivo cellular colonization [23]. The α-TCP used was of two types: (a) a fast hydration rate α-TCP (*CemContr*; by Mathys Medical, Switzerland); and (b) a slow hydration rate α-TCP (*Cem-N*; inlab preparation). Moreover, the slow hydration rate α-TCP was modified by sintering it again with 1, 8, and 24 wt% of iron citrate (*CemIC1*, *CemIC8* and *CemIC24*). Details have been published elsewhere [21]. On the other hand, the aqueous cement liquid phase was modified with 2.5 wt% disodium hydrogen phosphate (DHP; Na_2HPO_4; Panreac-131679). The liquid to powder (L/P) ratio was 0.32 ml/g. After mixing, the cement paste was immediately placed into disk moulds to ensure standardized shapes (10 mm diameter by 2 mm height). The cement disks were removed from the moulds after setting for 30 min in Ringers solution at 37°C. Immediately after, the disks were sterilized by UV-irradiation for 30 min and then used as substrates for cell culture.

2.2 Cytocompatibility testing

2.2.1 Cell culture

HEp-2 cells (epithelial cells derived from a human laryngeal carcinoma) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin/L-glutamine (Sigma Chemical Co., USA) in humidified atmosphere of 95% air, 5% carbon dioxide at 37°C. The medium was changed every 2 days. Cultures of 90% confluent cells were rinsed with phosphate buffered saline (PBS) and detached by incubating with trypsin/ethylenediaminetetraacetic acid (EDTA) (Sigma Chemical Co., USA). The detached cells were resuspended in fresh media and counted using a hemocytometer and 4% trypan blue as dye vital. The *HEp*-2 cells were seeded on top of cement samples at a density of 5×10^4 cells/well. Each cement disk was placed on the assigned well (24-well polystyrene-treated standard culture plate); before cell seeding, 200 µl of the culture medium was added into each well in order to minimize the number of cells attaching to the side and bottom's well or under the surface's disk. The wells containing cell culture medium without cement sample were used as controls (*Control-wells*).

2.2.2 Cell viability

To determine cell viability, the cells cultured on the cement substrates were evaluated after 1 and 6 days (1D and 6D) of incubation, using a quantitative MTT-assay. For this purpose, the medium in the wells was replaced with fresh medium and then 40 µl of MTT (3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye solution (3 mg/ml in PBS) was added on each well. After 3 h of incubation at 37°C, the medium was removed and formazan crystals (i.e. dark-blue insoluble product formed inside the viable cells, due to activity of mitochondria dehydrogenases) were solubilised with 500 µl of dimethylsulphoxide (DMSO) under continuous agitation (Mini Rocker MRI; Boeco, Germany) for 15 min, to facilitate the dissolution of the reacted dye. The liquid of each sample was removed for assay, which was performed in a 96-well plate. The absorbance of each well (i.e. the color intensity directly related to the number of viable cells) was read on a microplate reader (Anthos 2020 Microplate Reader; ASYS Hitech, Austria) at wavelength of 540 nm. The background absorbance produced by wells containing no liquid was subtracted from all the samples. The number of cells was determined using a linear equation obtained from a calibration curve containing absorbance values against different counts of cells. Cell relative viability (%) was calculated by $N_{test}/N_{control} \times 100$; where N_{test} is the number of cells corresponding to the tested sample and $N_{control}$ is the number of cells corresponding to the *Control-wells*.

2.2.3 Cell adhesion

The effect of the five experimental cement substrates on *HEp*-2 cells adhesion was determined as follows: the cells were cultured onto the cement disks for 1 and 6 days; the samples were washed with PBS to eliminate unattached or dead cells; cement disks were placed in new 24-well plate and evaluated by the MTT-assay following the experimental conditions described in the above sections. The *Adhesion profile* (*cells/cm*^{2}) was obtained by normalizing the number of cells adhered onto the cements to the disk-cement area available for cell's attachment.

2.3 Morphological study

Cells cultured for 1 and 9 days onto the experimental cement substrates and *Thermanox coverslips* (as positive cytocompatible control, *TMX*; Nunc, Sigma, Spain) were rinsed with PBS, fixed with 2.5% glutaraldehyde and subjected to graded alcohol dehydrations, and then sputter coated with gold. A scanning electron microscope (SEM, JEOL JSM-5610; Hitachi, Japan) was used to examine the cells onto disk-cements.

2.4 Statistical analysis

Each experiment was done in quadruplicate. The results were expressed as mean ± standard deviation (mean ± SD). A one-way ANOVA test was used to analyze the mean variance of the data. Tukey's multiple comparison was used to compare the data at a family confidence coefficient of 0.95. Statistical significance was accepted at a level of P-value < 0.05.

3 Results

3.1 Cytocompatibility

Figure 1 shows the *Cell relative viability* (%) of *HEp*-2 cells after 1 and 6 days of culture on iron-modified calcium phosphate cements (*CemIC1, CemIC8, CemIC24*), *Cem-Contr* and *Cem-N* substrates, which was quantitatively evaluated using the MTT-assay (see Sect. 2.2). After 1 day of culture, all the cements showed similar level of viability, ($P > 0.05$). The growth and proliferation on all the cements tested increased with the increase of culturing time. After 6 days of culture the viability of the cells cultured on *CemContr* was statistically significant ($P < 0.05$) as compared to *Cem-N* and *CemIC1*. Significant differences were also found among the *IM-CPCs*, i.e. *CemIC1* showed lower level of viability than *CemIC24* ($P < 0.05$).

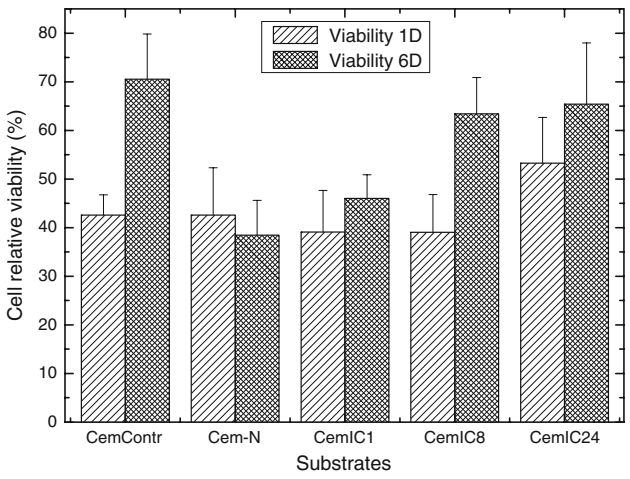

Fig. 1 Cell relative viability vs. cement formulations: effect of iron-modified cements on cells viability after 1 and 6 days of culturing (i.e. 1D and 6D)

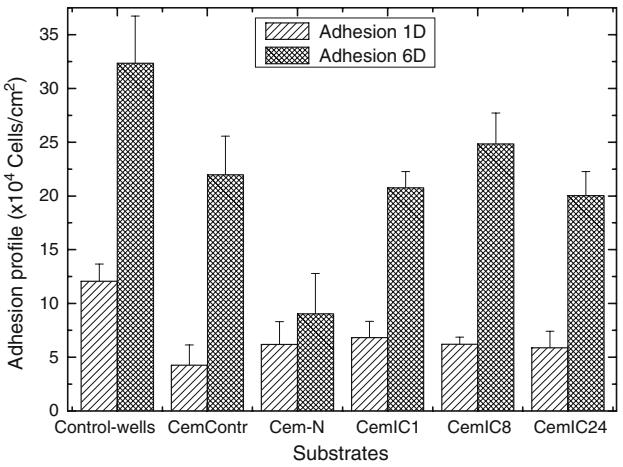

Fig. 2 Adhesion profile vs. cement formulation: effect of iron-modified cements on cells adhesion during 1 and 6 days of culturing (i.e. 1D and 6D)

Figure 2 shows the *Adhesion profile* (*cells/cm*^{2}) of *HEp*-2 cells onto *CemContr*, *Cem-N*, *CemIC1, CemIC8* and *CemIC24* substrates after cellular culture for 1 and 6 days. All the cements developed similar adhesion profiles after 1 day ($P > 0.05$). However, after 6 days, it was observed that the adhesion rate increased with the culture time with no significant differences among the iron-modified and the control (i.e. *CemContr)* substrates ($P > 0.05$). On the other hand, the non-active α-TCP control (i.e. *Cem-N*) showed no significant adhesion and proliferation results with the increase of the culture time ($P < 0.05$).

3.2 Cellular morphology

Figures 3–5 show the morphology of *HEp*-2 cells cultured onto *CemContr*, *CemIC1*, *CemIC8*, *CemIC24* and *TMX*, respectively, for 1 and 9 days. Cells cultured for 1 day had proliferated and the tendency to form a confluent cell monolayer was observed on all the cements, as shown in Fig. 3 (see left column). The cellular density qualitatively appeared similar on *CemContr* and *CemIC24*. These observations are in correspondence with the quantitative data in Fig. 2.

Figures 3 (see right column) and 4 show that cells attached and spread in a comparable manner on all the substrates, i.e. the cells developed many filopodia and large lamellipodia. After 1 day, the cells onto cements' surfaces had a flat appearance and showed similar bipolar and/or tripolar spindle-like morphology, seeming to attach on

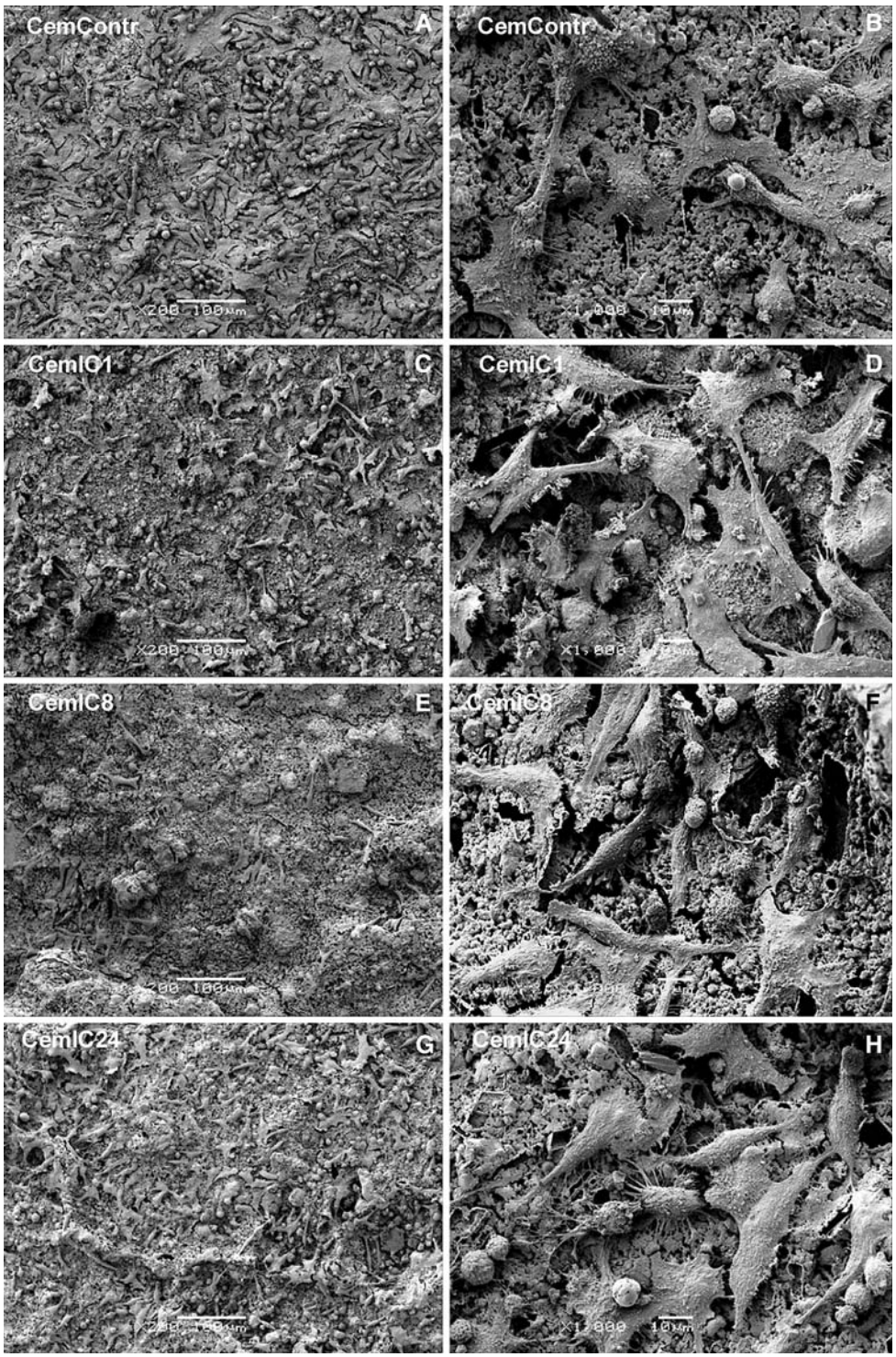

Fig. 3 SEM pictures of *HEp*-2 cells cultured for 1 day onto the experimental bone cements (see details on the text; left: 200×; right: 1000×)

specific surface's structural sites like sharp or ridge reliefs. A higher magnification is shown in Fig. 4, where cytoplasmic processes are observed attaching to the cement's surface. On the other hand, picture B in Fig. 4 (see top-right) shows cell–cell interactions on the cement's surface.

Figure 5 shows the results of cells cultured on *TMX* and *CemIC8* for 9 days. It was observed that some small areas were not covered by a confluent cell monolayer. This is characteristic for carcinogenic cells; i.e. after reaching maxima confluence some cells spontaneously detach. Figure 5d shows *HEp*-2 cells in the process of dividing. A higher magnification of cytoplasmic processes of *HEp*-2 cells anchored to the hydroxyapatite crystals of *CemIC8* surface is also shown in Fig. 5e–h).

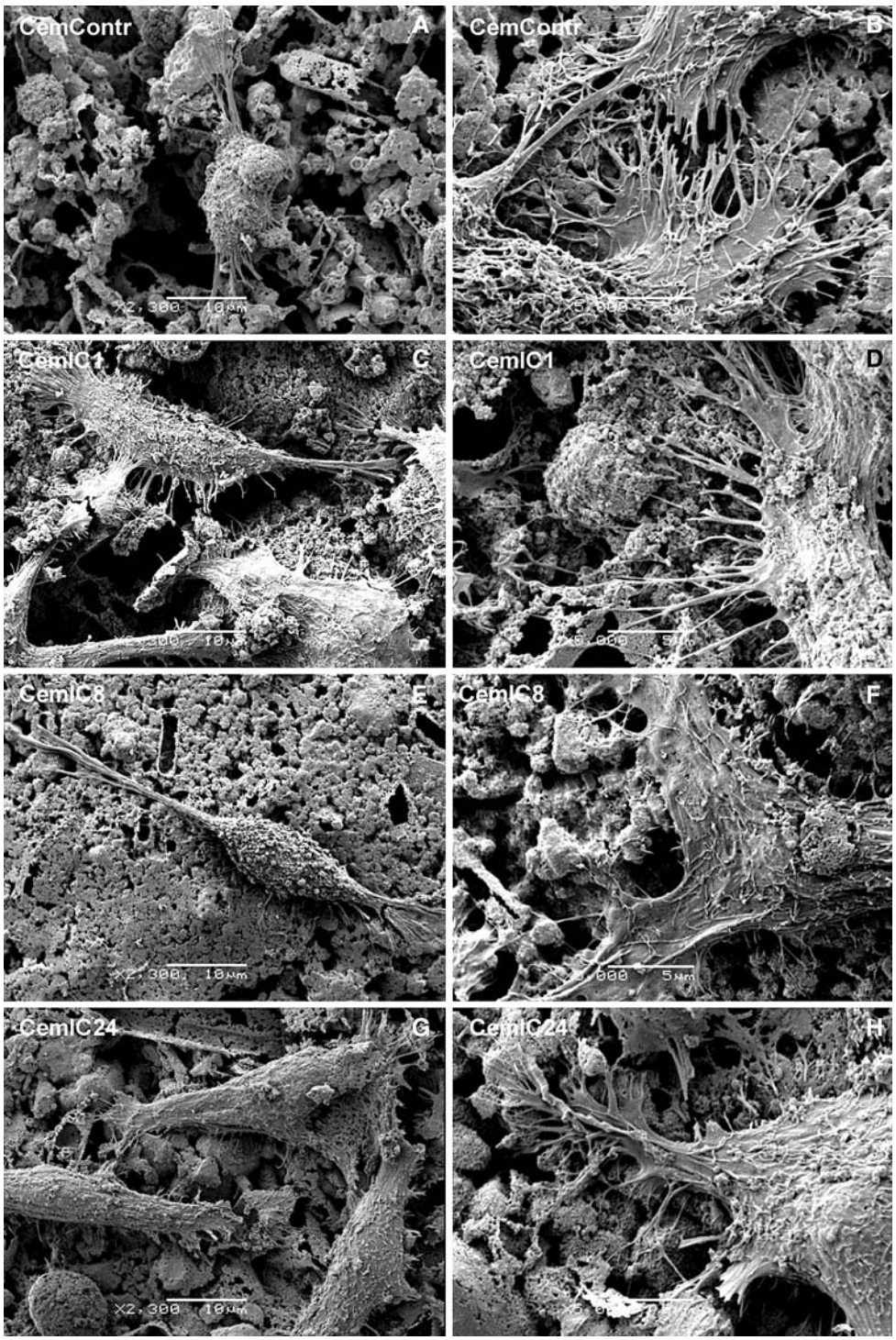

Fig. 4 SEM pictures of *HEp*-2 cells attached to the surface of the experimental bone cements after 1 day of culture (left: 2300×; right: 5000×). Image B shows cell–cell interactions (see details on the text)

4 Discussion

First of all, it should be highlighted that α-TCP is the main powder component of most commercial CPBC [12, 20] because its hydrates naturally into calcium deficient hydroxyapatite (CDHA; $Ca_9(HPO_4)(PO_4)_5OH$) during setting [11]. However, the hydration reaction of α-TCP into CDHA sometimes is limited (i.e. very slow rate-transformation) depending on uncontrollable ion impurities contained by the commercial reactants used during α-TCP high-temperature manufacturing [21]. For this reason, in this study, a cement control made with an active α-TCP (i.e. *CemContr*) and a cement control made with a non-active α-TCP (i.e. *Cem-N*) were used. As referred before,

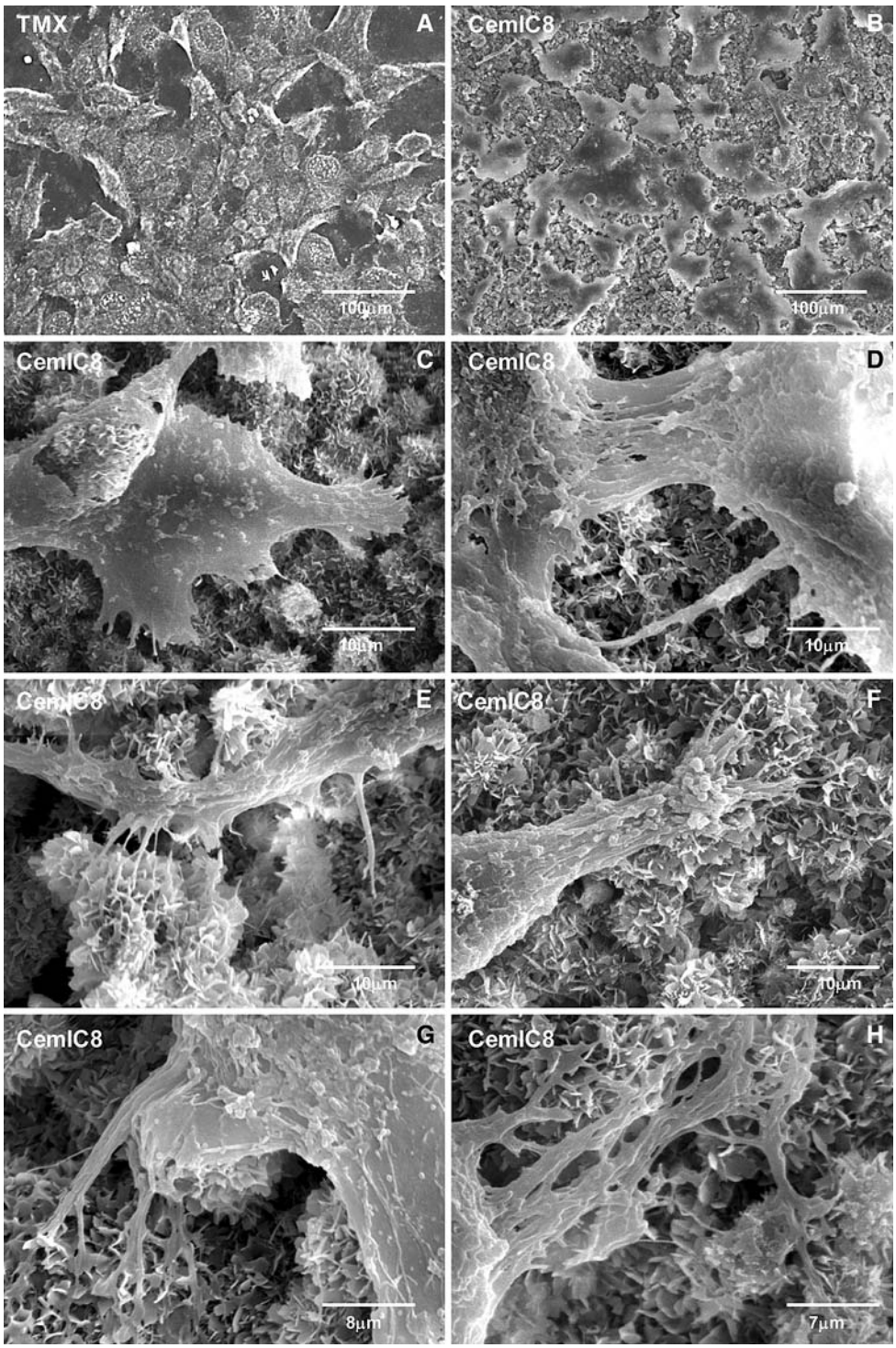

Fig. 5 SEM pictures of *HEp*-2 cells ocultured after 9 days on *TMX* (**a**) and *CemIC8* (**b**–**h**). Images **e**–**h** show the cytoplasmic process anchored to the hydroxyapatite crystals (see details on the text; (**a**) 370×; (**b**) 350×; (**c**) 3500×; (**d**) 4500×; (**e**) 5500×; (**f**) 6000×; (**g**) 7000×; (**h**) 8500×)

the resulting non-active α-TCP was modified in a previous study by sintering it again with IC in order to recover further α-TCP cement's setting properties [21]. In fact, IC minor additions did not change the α-TCP purity according to X-ray diffraction analysis (XRD; data not shown).

Taking into account these previous considerations, the results from Fig. 1 showed that the cellular viability of the iron-modified cements increased with the wt%-IC, i.e. iron modification of α-TCP did not show negative dose-dependent effects on *HEp*-2 cell's viability. On the other hand, the evaluation of the total iron liberated from the cements during curing time (data not shown) showed no iron-ion liberation phenomenon. This could be related to some stable fixation of iron into the structure of the α-TCP

phase (as indicated by XRD) as well as to the favored entrapment of iron into the apatitic phase formed during setting [24]. In fact, the setting and the hardening properties of α-TCP based bone cements are due to the progressive dissolution of the α-TCP particles, process which is followed by the nucleation of apatite crystals that growth until a stable mechanical structure of entangled apatite crystals is formed [11, 25]. Moreover, the viability results of Fig. 1 agree with the general delay of cells' growth observed as a consequence of initial failure of cells' attachment [26, 27] to the cements (as shown in Fig. 2), due to their surface's reactivity [28, 29] (i.e. continuous dissolution and precipitation reactions that affect calcium and phosphate ion concentrations during the cement setting). In addition, it should be point out that the cements used in this study contained also 30 wt% of CSD crystals, which after 5 days of cement incubation [23] can lead to interfacial supersaturated conditions due to their passive dissolution. In general, the behavior of the cells seeded on all the cements fit into hypothesis, except for cement *Cem-N*. In this case, both the cell attachment and the subsequent viability were lower than that observed for the other cements. It is thought that, in this case, the weak structural integrity of its surface (due to the slow hydration reaction of α-TCP into CDHA as confirmed by XRD analysis; data not shown) led to enough particle debridement as to favor cellular endocytosis. This ingestion process could adversely affect the calcium and phosphate homeostatic mechanism and cellular function. The chemical changes undergone by the cytosol and the subsequently accumulation of calcium into mitochondria could have promoted cell's death [30]. Moreover, actin cytoskeleton is molecularly linked to endocytosis [31] and the reorganisation of actin filaments could have negatively affected the cellular functions [32–34].

The results obtained from this study clearly demonstrated that the iron-modified apatitic cements showed similar initial cell attachment and viability profiles as the active α-TCP cement control (i.e. *CemContr*). Moreover, they had higher ability to enhance adhesion and proliferation of *HEp*-2 cells as compared with the non-active α-TCP cement control (i.e. *Cem-N*). This confirms that α-TCP iron modification is an improved way to recover not only the setting and hardening properties of α-TCP based cements [20], but also to recover their in vitro cytocompatibility. In fact, Fig. 2 shows, for the adhesion profiles after 1 day of culture, the same increasing tendency for both the whole iron-modified cements and the control (i.e. *CemContr*). Moreover, if we look at the adhesion profiles attained after 1 day of culture, these were slightly similar ($P > 0.05$) for all the substrates (i.e. around 48.61%) as compared to positive controls (i.e. *Control-wells*; considered as 100%). On the other hand, Fig. 2 also shows that the adhesion profile observed for *CemContr* after 6 days of culture was 87.35% of that of *Control-wells*. This difference on the initial cellular adhesion of cements as compared to controls could be attributed to the activity of the cement's surfaces (differences in pH and ion concentrations) as well as to differences in surface's morphology between the cements (i.e. rough crystalline surface) and the control-wells (i.e. smooth surface) [26]. In fact, it is known that the surface properties play an essential role on the first phase of cell-material interactions, including cell's attachment, adhesion and spreading on the biomaterial surface [35, 36]. The quality of this first phase depends on adhesion proteins [37], such as vitronectin and fibronectin, and will influence cell's ability to proliferate and subsequent viability. Despite the proliferative character generally exhibited by the neoplastic cells, the present in vitro cytocompatibility study was less favored by this feature because *HEp*-2 cells lack of production of fibronectin (adhesion protein) and tenascin-C (glycoprotein closely associated with cell proliferation and migration) [38].

Figure 3 also indicates that *CemContr* and *IM-CPCs* had similar initial cell's attachment onto their surfaces; i.e. most of them adopt a rather polygonal shape, which is a cellular feature indicative of better adhesion. Furthermore, the cell polarized morphologies sustain the locomotion phenomenon on cements' surface, which favored the colonisation of the surface, confirming the short-term biocompatibility of the experimental cements. Moreover, SEM pictures also show that the *IM-CPCs* substrates provided favorable conditions for further increase of cell's attachment (see Figs. 3 and 4). In fact, cement hydroxyapatite crystals provided anchorage for *HEp*-2 cells (see Fig. 5c–h) showing, as expected, that the microgeometry of a substrate plays a significant role in the whole process of cell's attachment and migration [36]. In this case, cytoplasmic processes allowed the movement of the migrating cells along the cement-like substratum and the tension generated by the forward movement explains the taut appearance of the cells (see Figs. 3 right column and 5), as observed also in other studies [39–41]. It should be noted that the surface's microgeometry of the *IM-CPCs* also provided favorable conditions for a rich network of cell-to-cell contact formation in the early stage of cell proliferation.

The results obtained in this study related to *IM-CPCs* and *Cem-N* were consistent with our previous results [20]. This confirms that iron modification of α-TCP is a useful approach to stabilize the mechanical properties of α-TCP based bone cements after setting without affecting its cytocompatility. Moreover, it is an interesting approach to look for some apatitic thermotherapeutic cements.

A final comment is needed to justify the use of epithelial cells instead of osteogenic cells in this study. The main reactant of the bone cement used in this research was

α-TCP. As stated at the beginning of this article, the α-TCP was modified during sintering with minor amounts of IC. It was not the objective of this research to study the osteogenic behavior of the new iron-modified bone cement which, on the other hand, is well known for α-TCP [9]. The interest was focused on the possible negative effect of the iron-ion solubility (oxidative damage) [6, 8] on the general mechanism of cellular adhesion and viability.

5 Conclusions

The present study showed that iron-modified α-TCP based bone cements have cytocompatible features. Both quantitative and morphologic evaluations showed that adhesion, proliferation and viability of *Hep*-2 cells were not negatively influenced by iron concentration in a dose-dependent manner. In fact, proliferation and cellular viability improved at the level of cement's surface and cement's environment. The results obtained show that iron-modified cements have the ability to support cellular colonization. The results might provide new insights into the development of new thermotherapy apatitic calcium phosphate bone cements. However, further in vitro and in vivo studies are required to confirm the possible application for bone repair/substitution. In fact, the positive effects observed in this study should be evaluated in detail with osteogenic cells in order to determine the levels of specific markers of the cellular function.

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References

1. K. Takegami, T. Sano, H. Wakabayashi, J. Sonoda, T. Yamazaki, S. Morita, T. Shibuya, A. Uchida, New ferromagnetic bone cement for local hyperthermia. J. Biomed. Mater. Res. Part B Appl. Biomater. **43B**, 210–214 (1998)
2. D. Arcos, R.P. del Real, M. Vallet-Regi, Biphasic materials for bone grafting and hyperthermia treatment of cancer. J. Biomed. Mater. Res. **65A**, 71–78 (2003)
3. M. Kawashita, S. Domi, Y. Saito, M. Aoki, Y. Ebisawa, T. Kokubo, T. Saito, M. Takano, N. Araki, M. Hiraoka, In vitro heat generation by ferromagnetic maghemite microspheres for hyperthermia treatment of cancer under an alternating magnetic field. J. Mater. Sci.: Mater. Med. **19**, 1897–1903 (2008)
4. C.C. Berry, S. Wells, S. Charles, A.S.G. Curtis, Dextran and albumin derivatised iron oxide nanoparticles: influence on fibroblast in vitro. Biomaterials **24**, 4551–4557 (2003)
5. E.H. Kim, H.S. Lee, B.K. Kwak, B.K. Kim, Synthesis of ferrofluid with magnetic nanoparticles by sonochemical method for MRI contrast agent. J. Magn. Magn. Mater. **289**, 328–330 (2005)
6. O. Kakhlon, Z.I. Cabantchik, The labile iron pool: characterisation, measurement, and participation in cellular processes. Free Radic. Biol. Med. **33**(8), 1037–1046 (2002)
7. M.W. Henze, M.U. Muckenthaler, N.C. Andrews, Balancing acts: molecular control of mamalian iron metabolism. Cell **117**, 285–297 (2004)
8. R. Cotran, V. Kumar, A.K. Abbas, N. Fausto, in *Pathologic Basis of Disease*, ed. by Robbins & Cotran, Chapt 1, 7th edn. (WB Saunders Co., 2004), pp. 14–17
9. S.V. Dorozhkin, Calcium orthophosphate cements for biomedical application. J. Mater. Sci. **43**, 3028–3057 (2008)
10. E. Fernández, F.J. Gil, S.M. Best, M.P. Ginebra, F.C.M. Driessens, J.A. Planell, Improvement of the mechanical properties of new calcium phosphate bone cements in the $CaHPO_4$-α-$Ca_3(PO_4)_2$ system: compressive strength and microstructural development. J. Biomed. Mater. Res. **41**, 560–67 (1998)
11. E. Fernández , Bioactive Bone Cements. in *Wiley Encyclopedia of Biomedical Engineering, 6-Volume Set*. ed. by M. Akay. (Wiley, USA, 2006), pp. 1–9
12. A. Gisep, R. Wieling, M. Bohner, S. Matter, E. Schneider, B. Rahn, Resorption patterns of calcium-phosphate cements in bone. J. Biomed. Mater. Res. **66**(3), 532–540 (2003)
13. D. Apelt, F. Theiss, A.O. El-Warrak, K. Zlinszky, R.B. Wolfisberger, M. Bohner, S. Matter, J.A. Auer, B. von Rechenberg, In vitro behavior of three different injectable hydraulic calcium phosphate cements. Biomaterials **25**, 1439–1451 (2004)
14. M. Bohner, Physical and chemical aspects of calcium phosphates used in spinal surgery. Eur. Spine. J. **10**, S114–S121 (2001)
15. S. Deb, J. Giri, S. Dasgupta, D. Datta, D. Bahadur, Synthesis and characterisation of biocompatible hydroxyapatite coated ferrite. Bull. Mater. Sci. **26**(7), 655–660 (2003)
16. D. Arcos, R.P. del Real, M. Vallet-Regi, A novel bioactive and magnetic biphasic material. Biomaterials **23**, 2151–2158 (2002)
17. M. Tyllianakis, D. Giannikas, A. Panagopoulos, E. Panagiotopoulos, E. Lambiris, Use of injectable calcium phosphate in the treatment of intra-articular distal radius fractures. Orthopedics **25**(3), 311–315 (2002)
18. M. Bohner, U. Gbureck, J.E. Barralet, Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials **26**, 6423–6429 (2005)
19. G. Lewis, Percutaneous vertebroplaty and kyphoplasty for the stand-alone augmentation of osteoporosis-induced vertebral compression fractures: present status and future directions. J. Biomed. Mater. Res. Part B: Appl. Biomater. **81B**, 371–386 (2007)
20. G. Lewis, Injectable bone cements for use in vertebroplasty and kyphoplasty: state of the art review. J. Biomed. Mater. Res. Part B: Appl. Biomater. **76B**, 456–468 (2006)
21. E. Fernández, M.D. Vlad, M. Hamcerencu, A. Darie, R. Torres, J. López, Effect of iron on the setting properties of α-TCP bone cements. J. Mater. Sci. **40**, 3677–3682 (2005)
22. M.D. Vlad, L.J. del Valle, M. Barracó, R. Torres, J. López, E. Fernández, Iron oxide nanoparticles significantly enhances the injectability of apatitic bone cement for vertebroplasty. Spine, (2008) (Accepted)
23. E. Fernández, M.D. Vlad, M.M. Gel, J. López, R. Torres, J.V. Cauich, M. Bohner, Modulation of porosity in apatitic cements by the use of α-tricalcium phosphate–calcium sulphate dehydrate mixtures. Biomaterials **26**, 3395–3404 (2005)
24. M. Jiang, J. Terra, A.M. Rossi, M.A. Morales, E.M.B. Saitovitch, D.E. Ellis, Fe^{2+}/Fe^{3+} substitution in hydroxyapatite: Theory and experiment. Phys. Rev. B **66**(22), 224107-1–224107-15 (2002)
25. E. Fernández, M.P. Ginebra, M.G. Boltong, F.C.M. Driessens, J.A. Planell, J. Ginebra, E.A.P. De Maeyer, R.M.H. Verbeeck, Kinetic study of the setting reaction of calcium phosphate bone cements. J. Biomed. Mater. Res. **32**, 367–374 (1996)
26. K. Anselme, M. Bigerelle, B. Noel, E. Dufresne, D. Judas, A. Iost, P. Hardouin, Qualitative and quantitative of human osteoblast adhesion on materials with various surface roughness. J. Biomed. Mater. Res. **49**, 155–66 (2000)

27. N. Ignjatovic, P. Ninkov, V. Kojic, M. Bokurov, V. Srdic, D. Krnojelac, S. Selakovic, D. Uskokovic, Cytotoxicity and fibroblast properties during in vitro test of biphasic calcium phosphate/poly-di-lactide-co-glycolide biocomposites and different phosphate materials. Microsc. Res. Tech. **69**, 976–982 (2006)
28. T. Suzuki, R. Ohashi, Y. Yokogawa, K. Nishizawa, F. Nagata, Y. Kawamoto, T. Kameyama, M. Toriyama, Initial anchoring and proliferation of fibroblast L-929 cells on unstable surface of calcium phosphate ceramics. J. Biosci. Bioeng. **87**(3), 320–327 (1999)
29. C.G. Simon, W.F. Guthrie, F.W. Wang, Cell seeding into calcium phosphate cement. J. Biomed. Mater. Res. **68A**, 628–639 (2004)
30. P. Juin, M. Pelletier, L. Oliver, K. Tremblais, M. Gregoire, K. Meflah, F.M. Vallette, Induction of a caspase-3-like activity by calcium in normal cytosolic extracts triggers nuclear apoptosis in a cell-free system. J. Biol. Chem. **273**(28), 17559–17564 (1998)
31. B. Qualmann, M.M. Kessels, R.B. Kelly, Molecular links between endocytosis and the actin cytoskeleton. J. Cell Biol. **150**(5), F111–F116 (2000)
32. C.S. Chen, M. Mrksich, S. Huang, G.M. Whitesides, D.E. Ingber, Geometric control of cell life and death. Science **276**, 1425–1428 (1997)
33. J.T. Ninomiya, J.A. Struve, C.T. Stelloh, J.M. Toth, K.E. Crosby, Effects of hydroxyapatite particulate debris on the production of cytokines and proteases in human fibroblasts. J. Orthop. Res. **19**, 621–628 (2001)
34. D.P. Pioletti, H. Takei, T. Lin, P. Van Landuyt, Q.J. Ma, S.Y. Kwon, K.L.P. Sung, The effects of calcium phosphate cement particles on osteoblast functions. Biomaterials **21**, 1103–1114 (2000)
35. K. Anselme, Osteoblast adhesion on biomaterials. Biomaterials **21**, 667–681 (2000)
36. C.C. Berry, G. Campbell, A. Spadiccino, M. Robertson, A.S.G. Curtis, The influence of microscale topography on fibroblast attachment and mobility. Biomaterials **25**, 5781–5788 (2004)
37. E.M. Harnett, J. Alderman, T. Wood, The surface energy of various biomaterials coated with adhesion molecules used in cell culture. Colloids Surf. B Biointerfaces **55**, 90–97 (2007)
38. T. Yoshida, E. Yoshimura, H. Numata, Y. Sakakura, T. Sakakura, Involvement of tenascin-C in proliferation and migration of laryngeal carcinoma cells. Virchows Archiv. **435**, 496–500 (1999)
39. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter (ed.), *Molecular Biology of the cell*, 4th edn. (Garland Science, NY, 2002), pp. 907–1080
40. B. Safiejko-Mroczka, P.B. Bell, Reorganization of the actin cytoscheleton in the protruding lamellae of human fibroblasts. Cell Motil. Cytoskeleton **50**, 13–32 (2001)
41. H. Lefaix, A. Asselin, P. Vermaut, J.M. Sautier, A. Berdal, R. Portier, F. Prima, On the biocompatibility of a novel Ti-based amorphouos composite: structural characterisation and in vitro osteoblasts response. J. Mater. Sci.: Mater. Med. **19**, 1861–1869 (2008)