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A New Medical Device Rigeneracons Allows to Obtain Viable Micro-Grafts From Mechanical Disaggregation of Human Tissues

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Autologous graft is considered the gold standard of graft materials; however, this approach is still limited due to both small amount of tissue that can be collected and to reduced cell viability of cells that can be obtained. The aim of this preliminary study was to demonstrate the efficacy of an innovative medical device called Rigeneracons® (CE certified Class I) to provide autologous micro-grafts immediately available to be used in the clinical practice. Moreover, Rigeneracons® is an instrument able to create micro-grafts enriched of progenitors cells which maintain their regenerative and differentiation potential. We reported preliminary data about viability cell of samples derived from different kind of human tissues, such as periosteum, cardiac atrial appendage biopsy, and lateral rectus muscle of eyeball and disaggregated by Rigeneracons®. In all cases we observed that micro-grafts obtained by Rigeneracons® displayed high cell viability. Furthermore, by cell characterization of periosteum samples, we also evidenced an high positivity to mesenchymal cell markers, suggesting an optimal regenerative potential.

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Recent research of non-embryonic stem cells isolation provides new possibilities for non-invasive procedures to rigenerate bone from stem cells collected from autologous tissues (Laino et al., 2006; Graziano et al., 2008a; Graziano et al., 2008b). The Rigeneracons® biologic tissue disgregator, based on Rigenera protocol, allows the extraction of micro-grafts of 50 μm from a few millimeters sample of autologous connective tissue directly within the surgery and immediately used without any manipulation or cell culture, as previously described (Graziano et al., 2007; Graziano et al., 2008c). If put in culture to test the nature and the composition of the micro-grafts, the latter show viable cells within them and the cell population endowed within the grafts is particularly rich in terms of progenitor cells. In this way, using this innovative medical device called Rigeneracons®, the patients are, in the same time, donor and acceptor of these micro-grafts. Currently, the main field of applications of Rigenera protocol are oro-maxillo-facial surgery and dermatology. To this regard, we recently demonstrated the efficacy of Rigeneracons® device in the obtaining progenitors cells from dental pulp (Brunelli et al., 2013) and adipose tissue recovered from the discard of follicular slicing (Zanzottera et al., 2014). Furthermore, we showed the Rigeneracons® device was able to provide autologous micro-grafts from connective tissues to improve the periodontal tissue generation (Graziano et al., 2013). On the basis of these evidences, we aimed to demonstrate in vitro the capacity of Rigeneracons® medical device to provide, from three different samples of human tissues, a high percentage of viable progenitors cells.

Conflicts of interest: The authors Trovato L and Graziano A declare conflict of interest because of they are in the Scientific Division of Human Brain Wave srl.

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Furthermore, we also characterize these progenitors cells by FACS evaluation.

Subjects and Methods

We performed experiments of cell viability and cell characterization on periosteum samples, cardiac atrial appendage biopsy, and lateral rectus muscle of eyeball. Periosteum samples were collected by three patients, who verbally informed and signed a consent form. All three patients underwent therapeutic dental avulsion and we collected periosteum samples from the palate (1 × 1 cm). The samples were immediately deposited in a sterile Falcon bottle and disaggregated under sterile conditions (vertical laminar flow hood) by Rigeneracons® medical device for 2 min. The cell suspension obtained was cultured and subsequently characterized by flow cytometry analysis (FACS) using a panel of antibodies which is usually employed to identify the mesenchymal cells, including CD90, CD105, CD73, CD45, and CD14, in addition to 7AAD staining in order to measure cell viability. We also obtained preliminary data of cell viability in cardiac cells derived from 20 different samples of cardiac atrial appendage biopsy and in the lateral rectus muscle of eyeball from three different patients affected by strabismus. The viability of cells obtained by cardiac atrial appendage biopsy was measured by automatic cell counting using Trypan blue to stain the cells obtained after mechanical disruption with Rigeneracons®, while the viability of cells derived by disaggregation of lateral rectus muscle was evaluated by FACS analysis using Propidium Iodide (PI) to marker the cells.

Results

After subculture of samples periosteum, we observed the appearance of the first cellular colonies after 15–20 days. One month after seeding, these cellular colonies were detached from the plate and re-seeded, and cells analyzed by confocal microscopy and flow cytometry. As indicated in the Figure 1, we evidenced two different cell populations, one from the elongated shape and one by rhomboid shape more large. We subsequently characterized the cell population at two different step: immediately after subculture and after one month, when the cellular colonies were reseeded. Cell characterization was performed by FACS and we reported that the cells obtained seem to belong to mesenchymal line as indicated by positivity for several mesenchymal cell markers, including CD90 (52%), CD105 (82%), and CD73 (82%) (Fig. 2A panels a–c). We also observed that these cells were negative for CD45 (Fig. 2 panel d) and CD14 (Fig. 2A panel e). Furthermore, staining the cells with 7AAD marker, we observed that cell viability was of 73% (Fig. 2A panel f). Similar results were reported when the cells were detached and reseeded (Fig. 2B panels g–l). In summary, these data indicate that these cells are positive to mesenchymal cell line markers and negative to hematopoietic and macrophages markers.

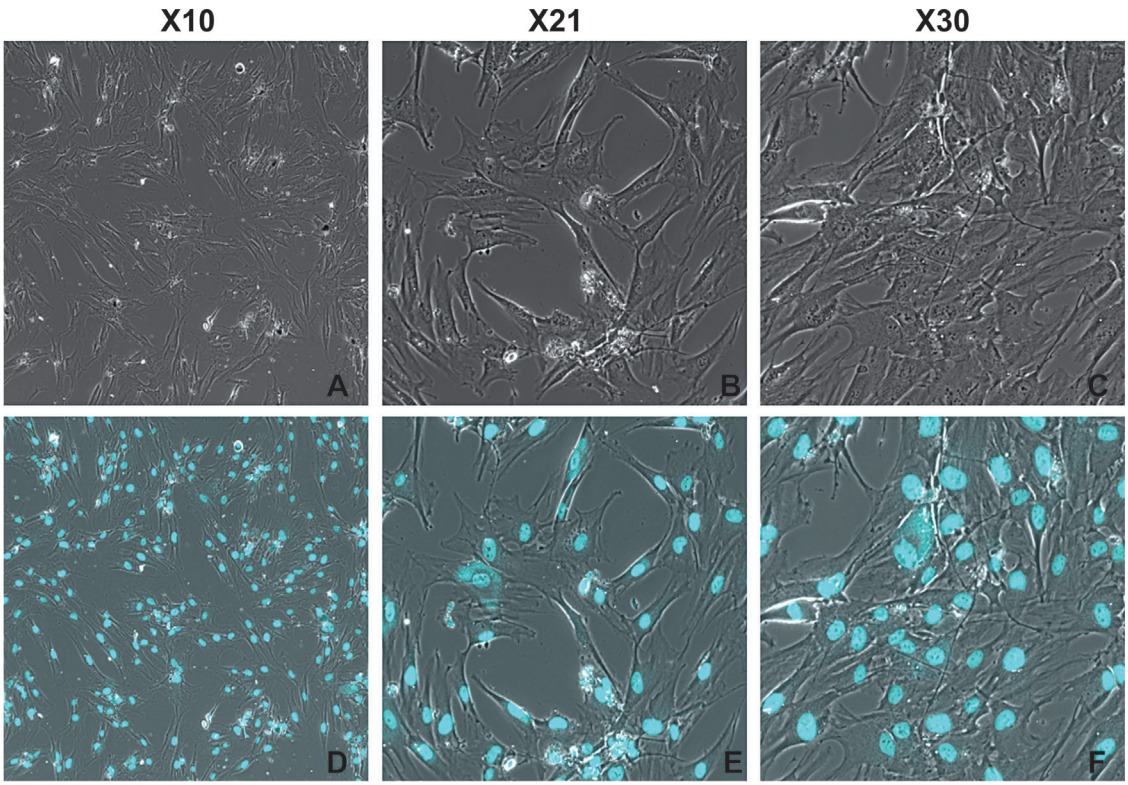

Fig. 1. (A–C) Images of cell culture obtained from periosteum sample after disaggregation with Rigeneracons® device. The cells were captured with confocal microscopy at different magnification (X10, X21, X30). (D–F). Nuclei were stained with 2 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI).

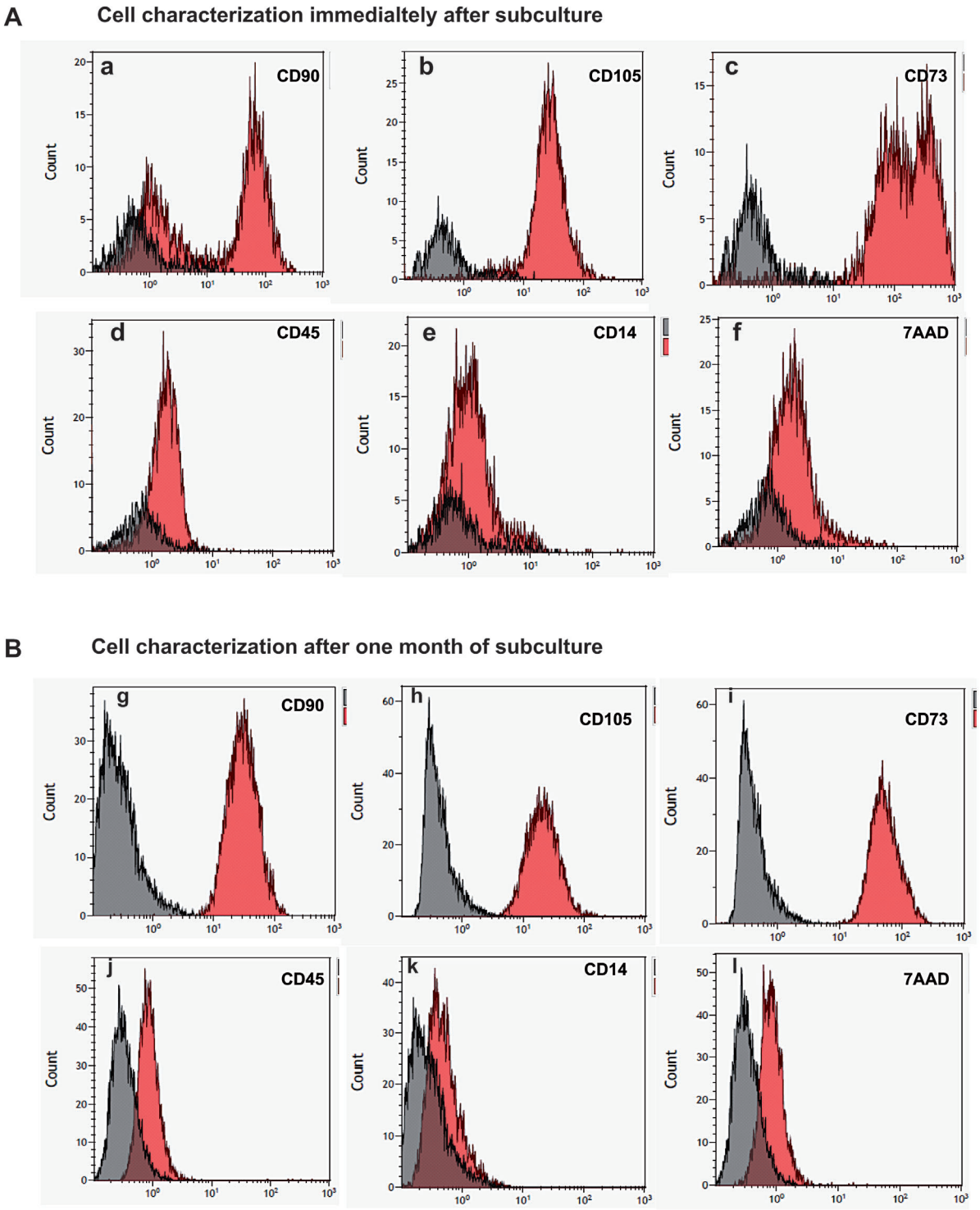

Fig. 2. Viability and characterization of cell isolated from periosteum. (A) FACS evaluation of one sample of periosteum immediately cultured after disaggregation with Rigeneracons® device. (B) FACS evaluation of the same sample cultured after one month and reseeded. Cell characterization was carried out using mesenchymal cell line markers, as CD90, CD105, and CD73. CD45 is a myeloid lineage marker and CD14 is a macrophage markers. Cell viability was whereas evaluated by 7AAD staining.

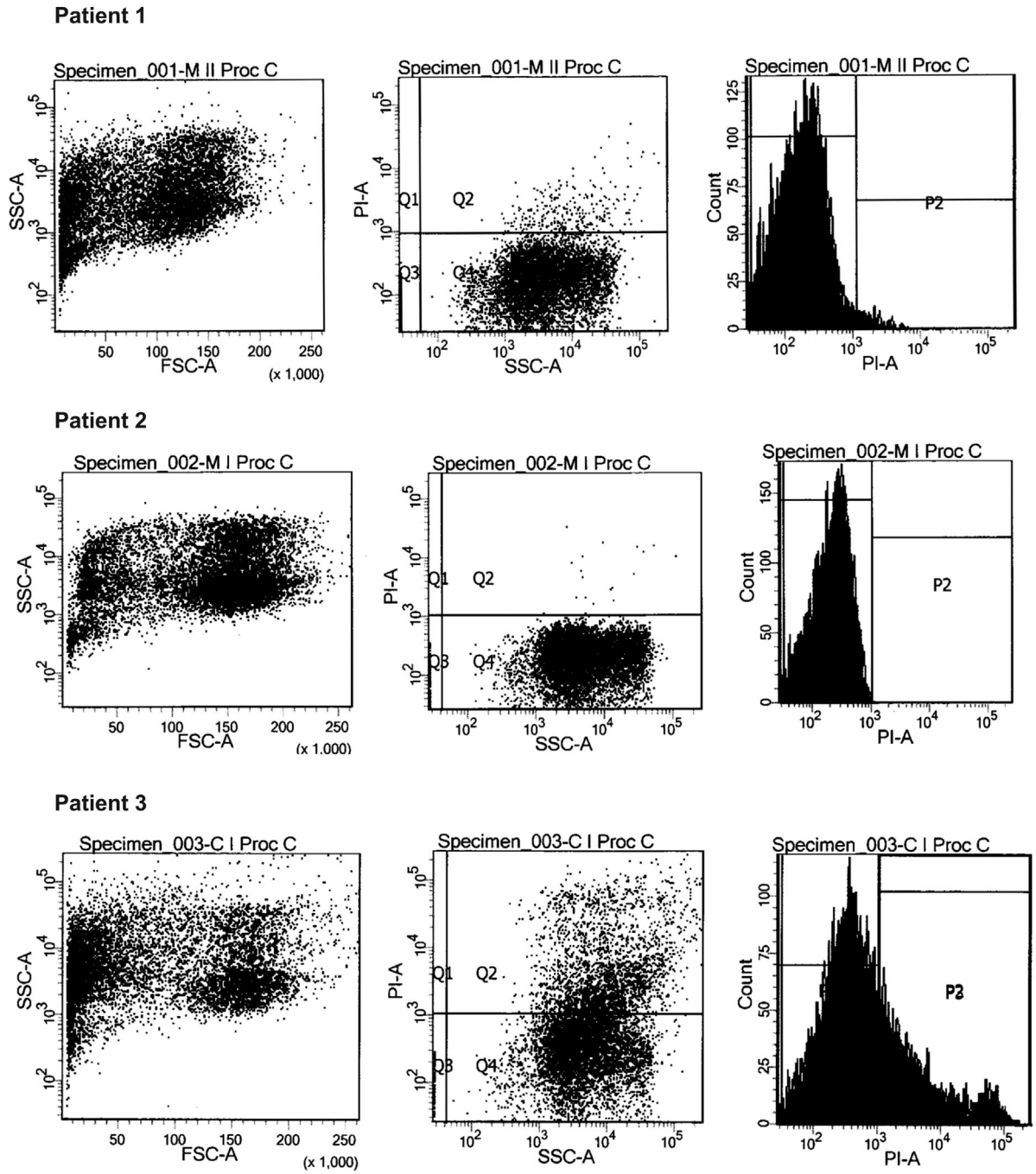

Fig. 3. Viability of cell isolated from lateral rectus muscle of eyeball. The image is representative of three samples analyzed and disaggregated with Rigeneracons® device. Cell viability was evaluated by FACS analysis using Propidium Iodide to stain the cells.

In addition to these data, we performed preliminary experiments of cell viability in different samples of atrial appendage tissue disaggregated with Rigeneracons® device. Also in this case was observed that viability of cells suspension obtained was of 96–100% (data no shown). In only one case, the cell viability was of 53%, likely due to the fact that tissue was not readily processed after dissection from the patient. The interesting data was that at room temperature, the cell viability was over 90%, also after 4 h (data no shown). Finally, we carried out very preliminary experiments in three samples of lateral rectus muscle of eyeball treated with Rigeneracons® device observing a cell viability of 97, 76, and 56%, respectively (Fig. 3).

Discussion

In this study, we reported our preliminary in vitro data on cell viability and cell characterization of progenitors cells obtained by a new medical device called Rigeneracons®. We in fact

showed that micro-grafts obtained by periosteum samples are enriched of progenitors cells maintaining the capacity to differentiate. In fact, cell characterization, performed by FACS, showed that these micro-grafts are rich of mesenchymal stem cells, that to date are mostly investigated in the tissue regeneration for their several biological properties (Baghaban Eslaminejad and Malakooty Poor, 2014; Sharma et al., 2014). In according to these results, Zanzottera et al. showed that percentage of pure progenitors (mesenchymal stem cells) was 15% and 17% in samples derived from the discard of follicular slicing respect to the percentage of 2% normally evidenced in literature. Furthermore, in the same paper, has been reported the high presence of endothelial cells and pericytes (Zanzottera et al., 2014) that in turn play a marked role in the tissue regeneration, promoting the post-traumatic revascularization.

It is evident that high viability is a pivotal factor in tissue regeneration in order to restore the correct anatomy and functionality of the same. The isolation of cells is often difficult and the methods of extraction, such as enzymatic digestion or mechanical disaggregation, require a time ranging from several minutes to a few hours, and this can be reduce their viability. Rigeneracons® device produces in a few minutes (about 2 min) a cell suspension containing millions of viable cells with a cut-off of 50 μm, opportunely selected by filtration. To this purpose, in this report we showed that percentage of viable cells derived by periosteum samples was very strong, suggesting that Rigeneracons® device provides an optimal and effective procedure of extraction. These data are in according to our previous data on derma samples, where we observed that cell viability was of 93% and 73% (Zanzottera et al., 2014). Optimal results on cell viability were also observed in the samples of cardiac atrial appendage biopsy and lateral rectus muscle of eyeball. The clinical efficacy of micro-grafts created by Rigeneracons® device was also demonstrated in an our very recent paper, where we reported an amelioration of management and wound healing of complex wounds in two different subjects (Giaccone et al., 2014).

Together to in vitro evidences, this new medical device provides other benefits, such as the easy and safety of Rigenera protocol during the time of intervention both in operating room or in ambulatory. Furthermore, using Rigenera protocol, the donor and acceptor are the same individual, preventing the possible complications linked to no autologous micro-grafts. Rigenera protocol is currently used in the oro-maxillo-facial field as showed by very recent studies (Brunelli et al., 2013; Graziano et al., 2013) and dermatology (Giaccone et al., 2014; Zanzottera et al., 2014) but it can be potentially used in others clinical field including plastic surgery and orthopedics.

In conclusion, we reported the high efficacy of Rigeneracons® device to provide a suspension of progenitors cells from different human tissues which display an high percentage of viability. In addition, we reported that samples derived from periosteum also present a good percentage of mesenchymal cells that, as well established, are able to differentiate in different cell type and improve the tissue regeneration.

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