FT-IR Characterization of Some Biological Materials Used in Reconstructive Surgery

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Biomaterials of different origins are essential for new many biomedical applications such as implants and prosthetics, pharmaceutical formulations, DNA and protein microarrays, drug and gene-delivery agents, or tissue engineering. In this paper we report on some characteristics of human tissues removed from abdominal area, which are currently used in the cranio-maxillo-facial reconstruction surgery (soft tissue correction). In fact, such adipose tissues are thought to be an abundant and accessible source of mesenchymal stem cells. However, little is known on their structure and composition. Fourier transforms infrared (FT-IR) and UV-visible spectroscopy, as well as fluorescence measurements proved to be useful techniques able to afford markers for different samples of graft tissue. Since the use of fat graft represents the future in the plastic and corrective surgery, such measurements should be taken into consideration for further investigations.

Keywords: FT-IR; fat graft; lipostructure; tissue formation; stem cells

Fourier transform infrared spectroscopy (FT-IR) proved to be a powerful technique of choice in medicine [1, 2], since it discriminates between the normal and pathological structures [3-5]. For example, FT-IR spectra of cancerous tissue in a certain organ differ from that of normal one in the same part of the body [6, 7].

A possible indication for this method can be the evaluation of the fat harvested from different sites of the body.

Neuber took in 1893 fat from a forearm and corrected facial flaws caused by bone tuberculosis [8]. In spite of excellent (immediate) post surgical results, necrosis caused by the lack of vascularization was soon after observed. However, an important improvement was made in 1926 when Miller modified the surgical technique, harvesting and reinjecting fat [9]. Unfortunately, the results were first disappointing because of the large rate of fat resorption.

At the end of the 1980's, Coleman developed the new fat harvesting and preparing technique, which he named lipostructure. The method was registered as trademark and then published in 1994 [10]. Lipostructure proved to be able to heal chronic wounds following radiotherapy or the diabetic foot [11]. The presence of pluripotent stem cells in fat graft are is thought to afford such good results. Recent papers have shown that mesenchymal stem cells (MSC), extracted from adipose tissue, are capable of forming cartilage, bone, muscle and fat [12, 13]. Lipoaspirates resulted from liposuction contain many growth factors, such as: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) [14]. These improve the transfer results and enable adipocytes to survive [15]. Despite the large use of the fat and the deep knowledge about all those sophisticated factors, little is known about the biomaterials used.

Therefore, this paper explores the hypothesis that the FT-IR might be a method of choice for investigating the differences between the grafts as well as characterizing the biomaterial removed from different patients with the aim to use it in facial reconstruction. Moreover, FT-IR spectra could be reliable evidence for improving the surgical procedure. In addition, such biomaterials have not been characterized in sufficient detail in literature, from both chemical and structural point of view.

Experimental part

Materials and methods

We used in this study analytical grade reagents, while all solutions were prepared with milliQ grade water with R = 18.2 Ω. Hexane was provided by Fluka. IR spectroscopy grade KBr was from Scharlau (Spain). The fat grafts were centrifuged in 1.5 mL Eppendorf tubes at 22°C and 3.000 rpm for 5 min. Samples were centrifuged using the Hettich MIKRO 22R cooling centrifuge (Hettich, Germany). The infrared spectra of tissue samples were recorded in solid KBr using a JASCO 660+FT-IR spectrophotometer (FT-IR Jasco Corporation, 2967-5, Ishikawa-cho, Hachioji, Tokyo 192, Japan). The FT-IR spectra were recorded in the frequency region 7000–400 cm^{-1}, under a resolution of 2 cm^{-1}, with a scanning speed of 2 mm sec^{-1}, and 20 scans per sample [16-18]. The UV-visible spectra were performed on a Libbra S35 PC UV/VIS spectrophotometer with 1-cm matched cells of quartz [19, 20]. The fluorescence spectra were recorded on a Kontron SFM-25 (Kontron Instruments SPA, Milan, Italy) instrument equipped with quartz cuvettes with a path length of 1 cm with total volume of 3 mL [21]. Samples were excited at λex = 260 nm.

Sampling and Procedure

The fat tissue was harvested from the periombilical and the external thigh regions. Lipoaspiration was completed

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with the help of standard techniques, as previously reported [22]. During the infiltrating stage, the donor area was instilled with analgesic solution (0,5% lidocaine) with 3 mm-diameter cannulas. Fat was harvested and stored in 5 mL syringes, then centrifuged at 3000 rpm for 5 min [23]. Three layers were evidenced in each syringe (fig. 1). The oil and blood fractions (superior and inferior layers) were discarded, as recommended by Viterbo and Ochoa [24]. The middle layer was also obeyed to spectroscopic analysis.

Results and discussions

FT-IR spectroscopy

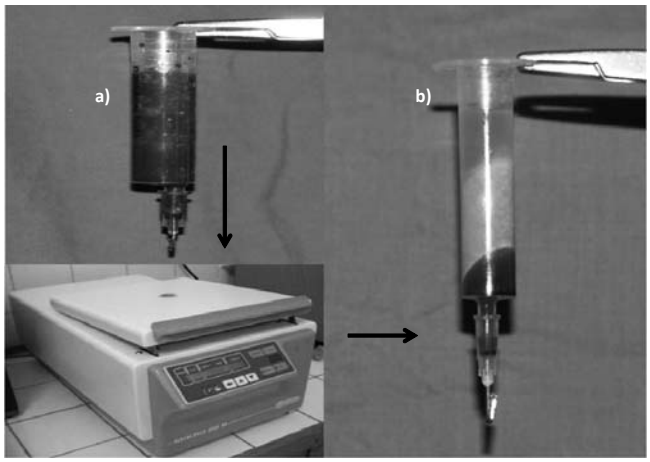

Fig. 1. Fat graft before (a) and after (b) centrifugation (the middle part is going to be used in reconstruction)

Figure 2 evidences the spectral differences between the two fatty tissue samples investigated in our work. Thus, the first sample (Fat graft 1) had a characteristic band in the „fingerprint" area of the infrared spectrum, at 726 cm^{-1}, whereas in the case of the second one (Fat graft 2), the similar band was more intense and shifted toward higher wavenumbers (732 cm^{-1}). Besides, the intense band at 1635 cm^{-1} from the first sample became a shoulder at the second one. However, the spectra of the two samples had much in common. For example, there is a common large band with a very characteristic shape in the region from 900 to 1400 cm^{-1}. Two extremely intense bands were found at 1456 cm^{-1} and 2926 cm^{-1}, respectively. Some other bands such as that at 1740-1750 cm^{-1} had different shape and maxima (1744 cm^{-1} versus 1747 cm^{-1}). While the peak groups around 2926 cm^{-1} had the same shape, the large band with a maximum at 3450 cm^{-1} from the first sample was completely different from the narrow band from the second one, which had a transmitance maximum at 3473 cm^{-1}.

Because we supposed that the biological samples taken for analysis may contain fat, especially palmitin, which is synthesized in high concentration in the cytoplasm alongside with albumin and other proteins, we have compared the spectra for graft tissues with those for palmitin and albumin. The comparative study revealed that the tissues were structurally more complex than fats and proteins they also contain. Moreover, FT-IR spectra could be „fingerprints" of such graft materials.

We extended the FT-IR measurements in the near infrared region (NIR), where some important bands around 5800 cm^{-1} were observed (fig. 3) and assigned to glycogen [25]. However, two more intensive bands were observed in NIR spectra, for the both samples, at 4333 and 4259 cm^{-1}, respectively, corresponding to a absorption energy equivalent with 12.43 kcal/mol and 12.22 kcal/mol. Nevertheless, the analyzed samples differ between them

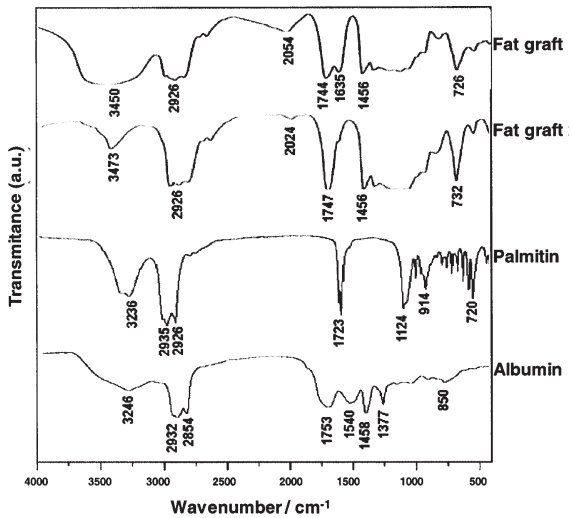

Fig. 2. FT-IR spectra of two fat tissue samples (Fat graft, 1 and 2) that are compared with those of palmitin (pure fat sample) and human albumin (protein sample)

by the presence of a large band with the maximum at 5192 cm^{-1} in the case of fat graft 1.

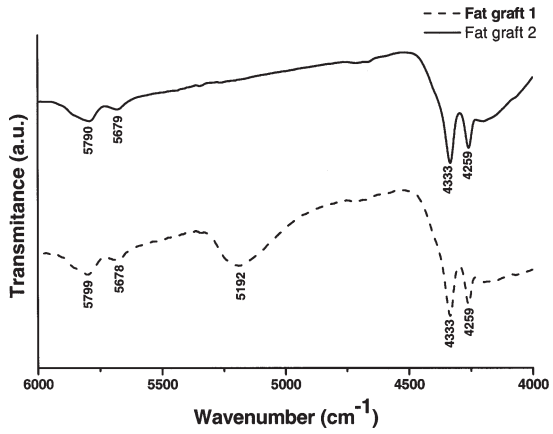

Fig. 3. Near infrared spectra of fat graft samples

UV spectroscopy

UV-visible absorption spectra displayed some characteristics bands in the ultraviolet region (fig. 4). The most intense peaks were fond at 208 nm in the case of fat graft 1 (relative absorbance intensity, RA, 0.632) and 205 nm (fat graft 2; RA 0.866), which suggest the presence of raw fat. However, the second sample also showed a small peak at 203 nm (RA 0.058), which can be assigned to the presence of aminoacids. A very large band was found in the case of both samples, which can be assigned to a complex of biological compounds, among them proteins might be the most prominent.

Nevertheless, UV-vis spectroscopy did not bring important evidence on the high complexity of the investigated biological samples [26, 27]. In addition, we used also a fluorimeter to study the fluorescence of these samples. However, no fluorescence was measured, but the samples solubilized in hexane proved to be opalescent. The turbidity was also evidenced by fluorimetry, when light skatering was observed (fig. 5). While fat was quikly solved in hexane, hidrophilic compounds (amino acids, proteins or even water traces) induced sample turbidity.

Sampling and procedure

In order to perform the spectroscopic measurements, we centrifuged fat graft samples at 3000 rpm for 5 min, since only the chemical components are necessary and not the intact fat cells. Recent articles have tackled the

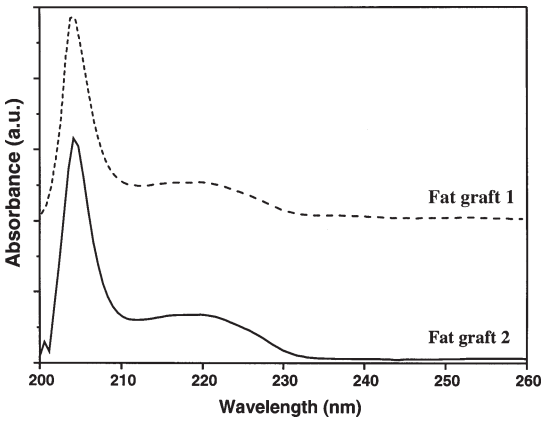

Fig. 4. UV spectra of hexane extracts from graft tissues.

topic of optimal centrifugal forces, cell viability, number of progenitor cells and density levels. Very high or low forces (3000 rpm or 500 rpm) either cause damage or present little effect. Many authors [20] thus concluded that the centrifugation at 1300 rpm results in good adipose tissue density, together with a good cell viability and possibility to preserve a large number of progenitor cells. Kim *et al*. [28] have conducted more experiments in order to establish the best centrifugation force for processing fat grafts; they concluded that centrifugation at 3000 rpm for 3 minutes is optimal, recommending it.

The Coleman technique produces more viable adipocytes and bears a better cellular function within the fat graft. The author uses special 3-mm cannulas for fat harvesting, called 'Coleman cannulas'. Rather than the conventional liposuction, most reports favor lipostructure as harvesting method [29].

Significant advantages and benefits of lipostructure are: (i) minimal invasiveness; (ii) local anesthesia and no need for hospitalization; (iii) minimal post operatory pains; (iv) capacity of integrating within the host tissues; (v) large amount of stem cells; (vi) reduced recovery period; (vii) product is autologous, so reject risk is inexistent; (viii) transferred fat maintains volume permanently; (ix) multiple donor sites. The ideal site for fat harvesting is the lower abdomen area, which was also investigated here.

Fortunately, a big advantage is the fact that adipose-derived stem cells (ASC) can be harvested in large numbers and they grow faster, compared to bone marrow derived stem cells. Also, no harm is done to the donor site [30]. All these characteristics make them a useful source of MSC, providing a promising future in the reconstructive surgery and cell-based therapy field [31]. Moreover, adult stem cells present no ethical issues concerning their usage, in contrast to embryonic stem cells [32].

Fat transfer seems to be a method with predictable, quite safe results and low complications. FT-IR technique may provide structural information on the large complexity of the samples taken from various patients. This technique revealed that the investigated biomaterial contains not only fat but many other biochemical compounds. FT-IR measurements indicated that each fat sample is quite different from others.

Perspectives

The investigated biomaterials contain living cells in which biostructure and its properties may be preserved [33]. The lipostructure technique can be successfully used curative, reparatory and aesthetic aspects, such as i) posttraumatic repairing of soft tissues, ii) correcting facial deformities as a result of tumor or cyst extirpation, iii) correcting facial relief deformed by congenital pathology;

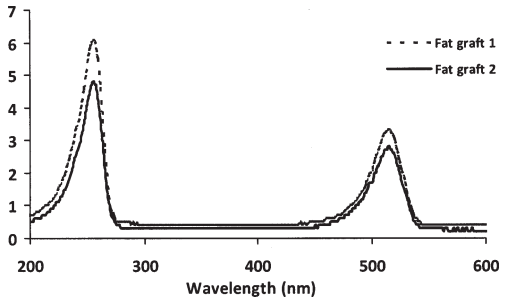

Fig. 5. Fluorescence spectra of extract fat grafts in hexane

iv) repairing scars, v) repairing tissue damage occurred after radiotherapy treatments and burns, vi) filling and attenuating facial wrinkles, and vii) repairing facial volume lost because of ageing. Our results suggest that a deep research on the biomaterials used may result in new applications and improvement of prognostic.

Conclusions

In this paper were investigated two fat graft samples using FT-IR and UV-vis spectroscopy in order to characterize them structurally and to differentiate such biomaterials by simple techniques. The two fat grafts proved to have completely different FT-IR and UV-vis spectra. A specific NIR absorption was observed, which was assigned to glycogen in the biomaterials analyzed. FT-IR spectra also revealed the high complexity of the investigated samples, which may contain stem cells, proteins, amino acids, glycogen, etc. Such spectra could be a simple and effective method for characterizing fat grafts. However, further research is needed to bring more information on such very valuable biomaterials.

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References

1.ZAHARIA, C., TUDORA, M.R., DAMIAN, C.M., VASILE, E., STANESCU, P.O., Mat. Plast, **50**, no. 3, 2013, p. 159.
2.GHIOCA, P., ROBU, S., PRISACARI, V., FILIP, V., SPURCACIU, B., IANCU, L., GRIGORESCU, R.M., Mat. Plast., **51**, no. 1, 2014, p. 94.
3.STANISZEWSKA-SLEZAK, E., FEDOROWICZ, A., KRAMKOWSKI, K., LESZCZYNSKA, A., CHLOPICKI, S., BARANSKA, M., MALEK, K., Analyst., **140,** 2015, p. 2273.
4.KHOSHMANESH, A., DIXON, M.W., KENNY, S., TILLEY, L., MCNAUGHTON, D., WOOD, B.R., Anal. Chem., **86,** No. 9, 2014, p. 4379.
5.GAREA, S.A., GHEBAUR, A., Mat. Plast., **49**, no. 1, 2012, p. 1.
6.WOOD, B.R., KIUPEL, M., MCNAUGHTON, D., Vet. Pathol., **51**, 2014, p. 224.
7.YANO, K., SAKAMOTO, Y., HIROSAWA, N., TONOOKA, S., KATAYAMA, H., KUMAIDO, K., SATOMI, A., Spectrosc., **17**, No. 2-3, 2003, p. 315.
8.RUBIN, J., JEWELL, M., RICHTER, D., UEBEL, C., Body Contouring and Liposuction, Saunders, 2012.
9.MILLER, C.G., Cannula Implants and Review of Implantation Techniques in Esthetic Surgery, The Oak Press, Chicago, 1926, p. 15.
10.COLEMAN, S.R., Clin. Plast. Surg., **24**, 1997, p. 347.
11.PITTEMGER, M.F., MACKAY, A.M., BECK, S.C., JAISWAL, R.K., DOUGLAS, R., MOSCA, J.D., MOORMAN, M.A., SIMONETTI, D.W., CRAIG, S., MARSHAK, D.R., Science, **284**, 1999, p. 143.
12.ZUK, P.A., ZHU, M., MIZUNO, H., HUANG, J., FUTRELL, J.W., KATZ, A.J., BENHAIM, P., LORENZ, H.P., HEDRICK, M.H., Tissue Eng., 7, 2011, p. 211.

13.ZUK, P.A., ZHU, M., ASHJIAN, P., DE UGARTE, D.A., HUANG, J.I., MIZUNO, H., ALFONSO, Z.C., FRASER, J.K., BENHAIM, P., HEDRICK, M.H., Mol. Biol. Cell., **13**, 2002, p. 4279.
14.PALLUA, N., PULSFORT, A.K., SUSCHEK, C., WOLTER T.P., Plast. Reconstr. Surg., **123**, 2009, p. 826.
15.ALHARBI, Z., OPLANDER, C., ALMAKADI, S., FRITZ, A., VOGT, M., PALLUA, N.,
J. Plast. Reconstr. Aesthet. Surg., **66**, 2013, p. 1271.
16.MURARIU, M., DRAGAN, E.S., DROCHIOIU, G., Int. J. Pept. Res. Therap.**, 15**, No. 4, 2009, p. 303.
17.HABASESCU, L., ZBANCIOC, G., GRADINARU, R., MURARIU, M., FERENCZ, L., DROCHIOIU, G., Rev. Roum. Chim., **58**, no. 6, 2013, p. 501.
18.ADOCHITEI, A., DROCHIOIU, G., Rev. Roum. Chim., **56**, no. 7, 2011, p. 783.
19.SURLEVA, A.R., DROCHIOIU, G., J. Chem. Educ., **90**, No. 12, 2013, p. 1654.
20.SURLEVA, A.R., DROCHIOIU, G., Food Chem., **141**, No. 3, 2013, p. 2788.
21.CIOBANU, C.I., HABASESCU, L., SCUTARU, D., DROCHIOIU, G., Lett. Org. Chem., **12**, No. 2, 2015, p. 91.
22.COSTAN, V.V., VICOL, C., DROCHIOI, C., BOIŞTEANU, O., POPESCU, E., Jurnalul de Chirurgie (Iaşi), **8**, No. 1, 2012, p. 53.
23.DUHOUX, A, CHENNOUFIA, M, LANTIERIA, L, HIVELIN, M., J. Plast. Reconstr. Aesthet. Surg., **66**, No. 7, 2013, p. 987.
24.VITERBO, F., OCHOA, J.S., Aesthet. Plast. Surg., **26**, 2002, p. 118.
25.LOMIWES, D., REIS, M.M., WIKLUND, E., Meat. Sci., **86**, No. 4, 2010, p. 999.
26.DROCHIOIU, G., MURARIU, M., PETRE, B.A., MANEA, M., PRZYBYLSKI, M., Rev. Chim. (Bucharest), **58**, no. 3, 2007, p. 311.
27.MURARIU, M., DRAGAN, E.S., DROCHIOIU, G., Biomacromolecules, **8**, No. 12, 2007, p. 3836.
28.KIM, I.H., YANG, J.D., LEE, D.G., CHUNG, H.Y., CHO, B.C., Aesthet. Surg. J., **29**, 2009, p. 35.
29.PU, L.L.Q., COLEMAN, S.R., CUI, X., FERGUSON, R.E.H., VASCONEZ, H.C., Plast. Reconstr. Surg., **3,** 2008, p. 932.
30.CASTEILLA, L., CHARRIERE, G., LAHARRAGUE, P., COUSIN, B., PLAMAT-BENARD, V., PERICAUD, L., CHAVOIN, J.P., Ann. Chir. Plast. Esthet., **49**, No. 5, 2004, p. 409.
31.MINTEER D., MARRA K.G., RUBIN J.P., Mesenchymal Stem Cells: Basics and Clinical Application, **129**, 2013, p. 59.
32.MIZUNO, H., J. Oral. Biosci., **55**, 2013, p.132.
33.MURARIU, M., DROCHIOIU, G., BioSystems, **109**, 2012, p.126.

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