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Recovery of Function in Anal Incontinence After Micro-Fragmented Fat Graft (Lipogems®) Injection: Two Years Follow Up of the First 5 Cases

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Abstract

Anal incontinence is common and significantly diminishes quality of life. This work aims to investigate the therapeutical potential of regenerative lipofilling by lipoaspirated fat, washed and reduced in size by the new device Lipogems®, in patients affected by chronic fecal incontinence.

5 patients with fecal incontinence due to obstetric injury and anorectal-pelvic surgery were followed up for 24 months after grafting of an average of 90 cc of aspirated and microfragmented fat (Lipogems®) in the external, internal anal sphincters and around pudendal nerves. Wexner-Incontinence-Score and Fecal-Incontinence-Quality-of-Life-Scale were used before and after treatment together with digital exploration, proctoscopy, endoanal ultrasound and anorectal manometry.

All patients observed an improvement both short and long term. The Wexner-Incontinence-Score improved from a preoperative mean of 14.0 to 3.4 at 3 months after treatment and remained stable up to 24 months. Anorectal manometry has reported over time significant improvements of pressure both at rest and in squeeze. Ultrasonography showed reabsorption of Lipogems tissue at 6 months and clear muscle repair at 12 and 24 months.

Our preliminary results suggest that this regenerative lipofilling can improve symptoms of fecal incontinence due to muscular and neural local trauma. Further studies are necessary to clarify the underlying mechanism, but a paracrine action of the mesenchymal stem cells contained in the fat tissue or in the purified fat cells themselves is postulated, as at 6-12 months when ecography of transplanted fat shows that the injected material disappeared while the beneficial clinical effects continue to be present and improve over time.

Keywords: Fecal incontinence, Lesions of anal sphincters, Mesenchymal stem cells, Regenerative therapy, Lipogems.

Introduction

Fecal incontinence is a disabling condition with a strong psycho-social impact that affects 2% to 15% of the general population, although its true prevalence is certainly underestimated[1,2]. It is generally caused by a variety of conditions and it often derives from a combination of factors; nevertheless the most common acquired causes are lesions of anal sphincters, often associated with damage to the peripheral innervation, due to obstetric injury, trauma, and anorectal-pelvic surgery[3-5]. Treatment options of severe incontinence include surgical procedures like sphincteroplasty, stimulated gracilioplasty and artificial sphincter insertion[6-10], while in mild and medium incontinence are usually reserved conservative interventions such as drugs, pelvic floor muscle training, biofeedback, and sacral nerve stimulation[11-12]. A minimally invasive alternative treatment is the local injection of bulking agents to increase the volume of sphincter apparatus. This procedure, which employs biological or synthetic materials, improves the pressure of anal sphincter, but it is severely limited because of the absence of dynamism and contractility of the materials which are also subject to migration and resorption. How-

ever, improvements in continence initially reported with the use of these techniques are not maintained by long-term[13-16].

Regenerative medicine is a great promise that provides hope for patients that display severe forms of degenerative or traumatic conditions, or for patients who are refractory to conventional treatments[17]. In this context, mesenchymal stem cells (MSCs) have been extensively studied with regard to their potential therapeutic applications. MSCs are multipotent adult stem cells capable of self-renew and differentiation potential, found in bone marrow (BM-MSCs) and in adipose tissue (ADSCs)[18]. Furthermore MSCs produce secreted factors such as cytokines and chemokines, or growth factors, which mediate diverse functions by cross-talk between different cell types, stimulating reparative responses[19,20].

Recent studies of regenerative medicine have revealed that BM-MSCs are able to stimulate the host to differentiate into mature cells of various tissues[21]. In particular, BM-MSCs injected into skeletal muscle generate favorable conditions for the growth of muscular cells by virtue of their paracrine activity[22-24].

Furthermore, recent experimental investigations *in vitro* and *in vivo* have reported improved regeneration of peripheral nerves with the use of BM-MSCs[25-28]. Because of encouraging use of MSCs to repair muscle damage in animal models with induced urinary[29,30] and anal incontinence[31,32], and, on the basis of encouraging results by clinical studies in patients with urinary[33,34] and anal incontinence [35], we considered of employing microfractured and washed adipose tissue with remarkable regenerative properties (Lipogems®)[36-39] to repair lesions of the anal sphincters in adult patients with fecal incontinence. This new procedure, derived from lipofilling surgical technique[40-42], represents a new field of regenerative surgery whose therapeutic criteria are based on the possibility of transferring the medicinal cells that are normally present in several tissues of adult human body, such as bone marrow, dental pulp, and adipose tissue[43-45], in those damaged areas of the human body that require repair.

Moreover MSCs isolated from adipose tissue, compared to bone marrow, present some peculiar characteristics. 1 g of adipose tissue contains 500 times more MSCs than 1 g of bone marrow aspirate[46]. Indeed, besides showing phenotypic and transcriptional profiles similar to that of the other MSCs, ADSCs express CD34 glycoprotein[47], stromal markers and endothelial cell markers[48]. Moreover, ADSCs have a higher yield upon isolation and a greater proliferative rate in culture when compare with MSCs isolated from other sources[49] and possess restorative abilities that make them a good candidate for regenerative therapy[50].

The adipose tissue extracted from humans by a gentle liposuction was treated through the use of mechanical processes of microfracturing, filtering and washing in a complete closed system. The healing potential of the MSCs contained in the adipose tissue is maintained by avoiding the use of enzymes and/or any other additives. Cellular biology studies have revealed that Lipogems® product is composed of small adipose tissue spherical clusters with micro-fragments of intact connective structure maintained viable by vascular support, particulary rich in pericytes and MSCs exposed on the surface of the vascular stroma[37,39]. Several investigators have hypothesized that pericytes are the precursors of the MSC, and their protruding position into the endothelial lumen of blood vessels makes them ready to monitor and react to local or systemic signals[51-55]. These properties contribute to make Lipogems® able to survive in a tissue and facilitate engraftment when autologously inoculated in target tissues[36].

Materials and methods

Ethics Statement and samples collection

The study was performed according to the Declaration of Helsinki. From June 2011, 5 consecutive patients (4 females, 1 male) aged from 46 to 74 (median 61 years), with at least 12 months history of fecal incontinence to liquids and/or solid stool confirmed by incontinence diaries kept by patients for two week, were treated to correct fecal incontinence with injection of Lipogems® into anal region. Fecal incontinence resulted from anal sphincter injury, peripheral nerve damage, or a combination of both, as shown in Table 1. After treatment, patients were followed-up for at least 2 years. All patients were advised about the potential benefits and risks and gave informed consent to the study approved by the local Ethics Committee. Before the Lipogems procedure, all patients have attempted medical and rehabilitation treatments with unsatisfactory results. Moreover, oncologists have provided a consent for the treatment in patients with previous history of cancer.

Table 1. Clinical features of the patients.

Patient	History	Wexner score (0>20)	Endoanal ultrasonography	Ano-cutaneous reflex	Anorectal manometry (values in mmHg)
A female age 61	At the age of 27 difficult birth with laceration and episiotomy	11	Combined ruptures of **EAS** and **IAS** localized in the anterior area of the muscular rings in the middle and upper portion of anal canal	Normally present	Hypotonia at resting pressure, low pressure increasing in squeeze *Mean resting pressure = 14* *Maximum resting pressure = 30* *Mean squeeze pressure = 33* *Maximum squeeze pressure = 56*
B male age 61	At the age of 55 anterior resection of the rectum with coloanal anastomosis, followed by radiotherapy and chemotherapy for rectal cancer	14	Diffuse lesions of the **EAS** and **IAS** with hypo-and hyper-echogenic areas related to fibrous regression	Impaired	Hypotonia at resting pressure, low pressure increasing in squeeze *Mean resting pressure = 23* *Maximum resting pressure = 28* *Mean squeeze pressure = 31* *Maximum squeeze pressure = 76*
C female age 65	Aged 50 to 56 repeated pelvic surgery and chemotherapy for ovarian carcinoma	16	No evidence of injury of the anal sphincters	Absent	Resting pressure within normal limits, low pressure increasing in squeeze *Mean resting pressure = 43* *Maximum resting pressure = 46* *Mean squeeze pressure = 55* *Maximum squeeze pressure = 84*
D female age 46	Sacral fracture in childhood. At the age of 34 difficult birth with laceration and episiotomy.	13	Combined ruptures of **EAS** and **IAS** localized in the anterior area of the muscular rings in the middle and upper portion of anal canal	Normally present	Hypotonia at resting pressure, low pressure increasing in squeeze. *Mean resting pressure = 14* *Maximum resting pressure = 20* *Mean squeeze pressure = 27* *Maximum squeeze pressure = 51*
E female age 74	Unreported significative data	16	Combined ruptures of **EAS** and **IAS** localized in the anterior area of the muscular rings in the middle and lower portion of anal canal	Absent	Hypotonia at resting pressure, low pressure increasing in squeeze. *Mean resting pressure =30* *Maximum resting pressure = 35* *Mean squeeze pressure = 39* *Maximum squeeze pressure = 49*

External anal sphincter (EAS), internal anal sphincter (IAS)

Diagnostic procedures

Prior to treatment, anal incontinence was evaluated using the Wexner Incontinence Score[56] and the quality of life was assessed with Fecal Incontinence Quality of Life (FIQL) Scale[57]. Patients were also subjected to digital anorectal examination and were investigated with proctoscopy and anorectal manometry (Medtronic Polygram – water perfused 4 channel catheter). Furthermore, endoanal ultrasound was performed to evaluate lesions of the anal sphincters using a 10-MHz rotating endoprobe (Kretz ERW7-10AKP – Combison 401 GE Medical Systems). Lipogems® treatment was conducted with patient placed in lithotomic position under general anesthesia, in a single surgical session that includes 3 different steps: 1) Harvesting of adipose tissue from the patient; 2) Processing of adipose tissue with Lipogems® device; 3) Re-inoculation of the product in the same patient.

Lipogems treatment

1. **Harvesting of adipose tissue**
   The lower or the lateral abdomen were chosen as donor sites for fat graft harvesting using a technique derived from traditional surgical liposuction. A blunt 13G cannula with multiple elliptical holes of 1x2 mm distributed along it, was employed for fat suction. This disposable cannula, connected with a 10 cc syringe, allows a fast and a traumatic suction of fat with few cannula strokes. Harvesting of fat is done 10 minutes after injection of saline and 1:500000 adrenaline in the selected area.

2. Processing of adipose tissue with Lipogems® device

The aspirated fat was immediately processed in the Lipogems® closed system (Figure 1). This disposable device progressively reduces the size of the fragments of adipose tissue from spheroidal cluster with a diameter from 2.5 to 3.5 mm to a micro-cluster from 400 to 900 μm, while eliminating oily substances and blood residues which have pro-inflammatory properties. The mechanical processes of microfracturing, washing and filtration take place in a system completely immersed in physiological solution avoiding the presence of air, in order to make possible the reduction in volume and minimize any traumatic action on cellular products. The system allows harvesting Lipogems® microfractured adipose tissue rich in pericytes and MSCs which are naturally present in adipose tissue niche[37-39,58]. The purified Lipogems fat tissue was collected in a 60 cc syringe and positioned to gently decant by gravity in order to remove excessive saline solution. Then the product of lipogems tissue is transferred in several 1 cc syringes to be re-injected in the same patient.

3. Re-inoculation in the patient

On endoanal ultrasound guidance, internal and external anal sphincter were identified (Figure 2). Using a special disposable 19G cannula with blunt tip, Lipogems® tissue was inoculated in the muscle defects (Figure 2a), in the intersphinteric space (Figure 2b) and all around circularly in the remaining portions of the external anal sphincter (Figure 2c). Luer lock syringes ranging from 1 ml are used to inject the ideal amount of tissue in each treatment. In *patient B, C, and E*, where it was evident a various degrees of deficiency innervation of the sphincter muscles, Lipogems® was also injected along the course of the pudendal nerve with a transperineal technique ultrasound-guided[59]. The fat micrograft was injected in multiple tunnels into the tissues taking care to deposit it in small quantities (1 ml) in only one place each time, from distal to proximal.

After the procedure, postoperative pain was evaluated by compilation of a Visual Numeric Scale (VNS, score 0-10) by the patients. All patients were re-investigated at 3, 6, 12, 18 and 24 months from the treatment to revaluate the physiological contractility of the anal sphincters, the symptoms of fecal incontinence, the quality of life and the sonographic features in the site of inoculation of the Lipogems® by repeating the same preoperative examinations. The surgical procedures were performed by A.G. and C.T. All the diagnostic examinations, preoperative and postoperative were performed by A.G. and by another independent investigator R.V.

Statistical analysis

The results are expressed as mean ± SD (standard deviation). The statistical significance was determined by the two-tailed Student's *t* test. Differences were considered statistically significant at $p<0.05$.

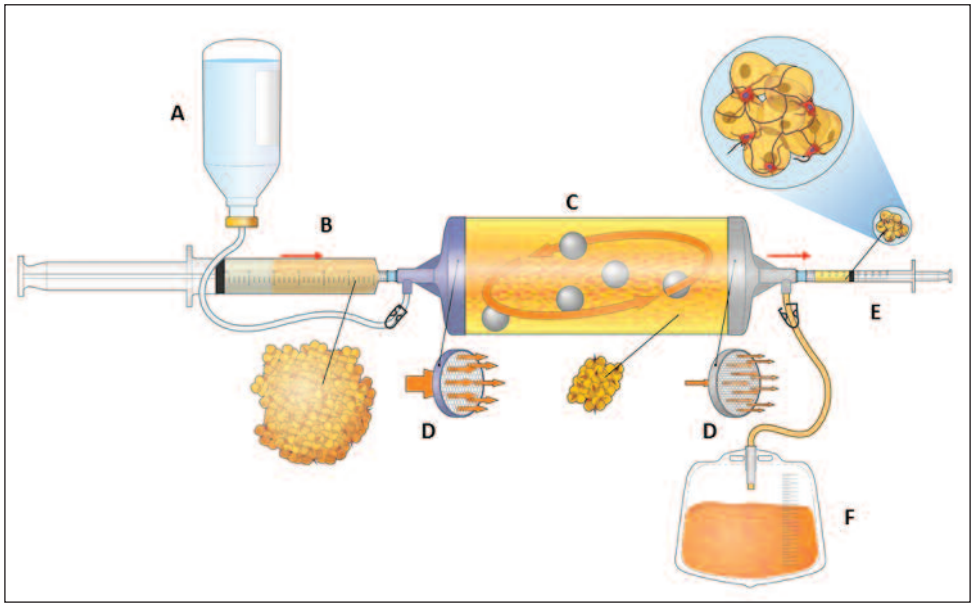


Figure 1. Lipogems device. Lipogems device is a complete closed system filled with physiologic solution. It allows the reduction of the size of lipoaspirate clusters after washing of oil, blood and cellular debris. *A*, Sac with physiologic solution; *B*, Syringe with lipoaspirate clusters; *C*, Washing chamber containing marbles for the emulsion of fluid and elimination of oil and blood against gravity; *D*, Mechanical filters; *E*, Syringe with clusters of reduced size; *F*, Sac with waste oil and blood.

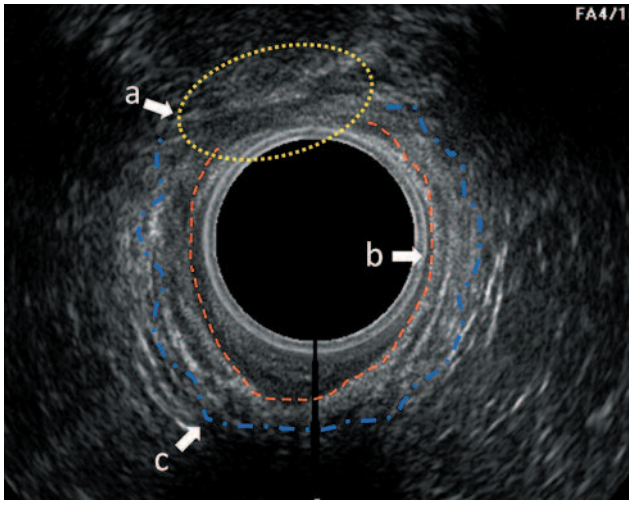


Figure 2. Lipogems injection. Areas of inoculation of Lipogems (endoanal ultrasound image at mid anal canal level). *a*, defect of external and internal anal sphincter; *b*, intersphinteric space; *c*, peripheral zone of the external sphincter.

Results

Surgical procedure

In all 5 patients, the procedure was successfully completed without intraoperative or postoperative complications. The median operation time, including the three different phases of harvesting, processing and reintroduction of the substance, was 73 minutes with most time used for reinjections. An average of 330 cc of lipoaspirate was collected from each patients. As a result of processing with the technique Lipogems®, the average amount of product obtained, ready for injection in patients, was 90 cc. During procedures, no problem of abnormal accumulation of Lipogems®, nor needle clogging, occurred.

Quality of life

Patients have well tolerated the procedure and the median postoperative pain evaluated by VNS (score 0-10) was 1.4, mainly referred to the site of abdominal fat suction. The postoperative hospitalization was 1 day in all patients. Patients' satisfaction for the treatment was very good and the median Fecal Incontinence Quality of Life (FIQL) score for all patients has increased from preoperative values. After 3 months from treatment, lifestyle improved from 1.8 to 3.4, coping from 1.6 to 3.6, depression from 2.1 to 3.4 and embarrassment from 1.9 to 3.5, remaining quite stable for the 2 years of follow up as shown in Figure 3.

In the 5 patients we reported a significant improvement in the average values of the overall Wexner Incontinence Score that has changed from a preoperative value of 14.0 to value of 3.4 at assessment three months after treatment, to value of 4.0 at 6 months and to value of 4.4 at one year. During the second year of follow up values remained stable with a moderate improvement trend, recording values of 4.2 at 18 months and 4.2 at 24 months (Figure 4). Moreover, the comparison of Wexner Incontinence Score measured pre-implantation and at 3, 6, 12, 18, 24 months in all 5 patients indicates a significant reduction in the Wexner score that decrease by a mean of 14.0 units to 3.4 units at 3 months and remained stable over the time course of our study (Figure 5).

Manometric findings

Global improvement was also reported by manometric findings. After treatment, the overall mean values of the anal pressure at rest and in squeeze recorded by anorectal manometry was improved in all the five patients as reported in the graph in Figure 6. Indeed, the

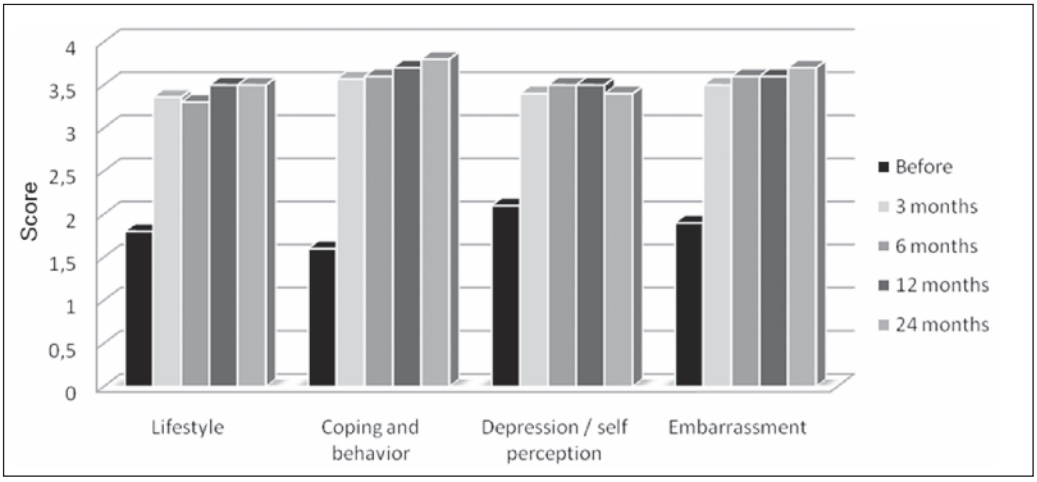


Figure 3. Fecal Incontinence Quality of Life Score. Changes in Fecal Incontinence Quality of Life score in 5 patients before and 3, 6, 12, 24 months after Lipogems treatment.

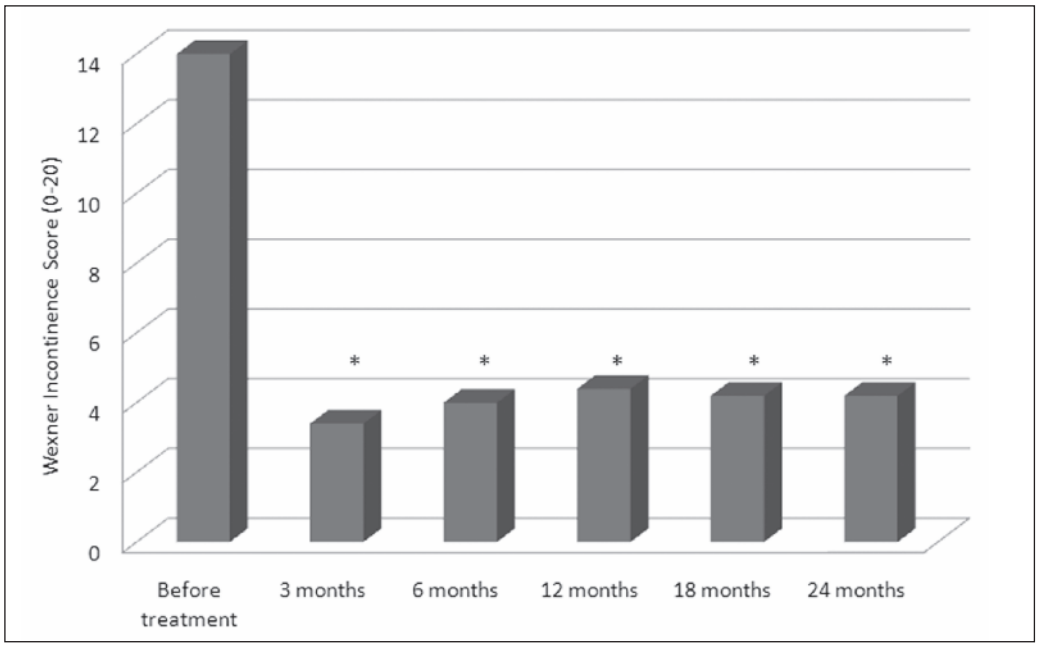


Figure 4. Wexner Incontinence Score. Average values of the Wexner Incontinence Score (0-20) for all the enrolled patients before and after Lipogems treatment. *$p<0.05$.

mean value at rest of 24 mmHg and in squeeze of 39 mmHg reported before regenerative therapy, increased respectively to 34 mmHg and 80 mmHg at 3 months, to 38 mmHg and 90 mmHg at 6 months, to 35 mmHg and 96 mmHg at 12 months, to 52 mmHg and 117 mmHg at 18 months and to 45 mmHg and 112 mmHg at 24 months.

Similar results were given for maximum resting and squeeze pressures that increased, respectively, from a median of 38 and 72 mmHg at baseline to 58 and 112 mmHg at 3 months, to 56 and 120 mmHg at 6 months, to 48 and 137 mmHg at 12 months, to 64 and 172 mmHg at 18 months and to 63 and 170 mmHg at 24 months after the procedure (Figure 6).

Interesting, a clear increase in the anal contraction pressure was recorded for all the patients 12 months after the treatment. Indeed, the contract ability of the sphincters increase significantly after Lipogems® treatment (Figure 7).

Physical examination

3 months after treatment the sphincters relaxation previously detectable in all patients was no longer appreciable replaced by an effect of filling in the anal canal whose walls appeared soft and elastic at finger palpation. From 6 to 12 months was clinically evident a reduction of filling in the anal canal which was maintained up to 24 months. At the same time

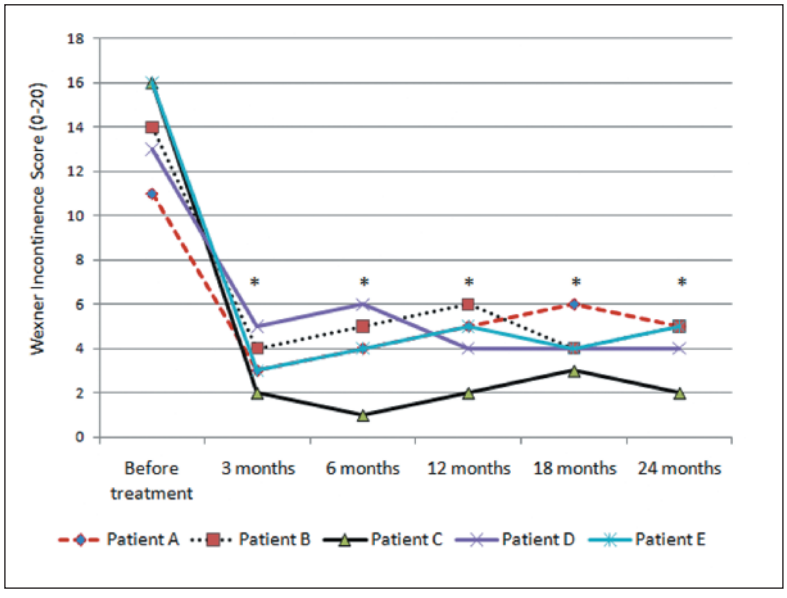
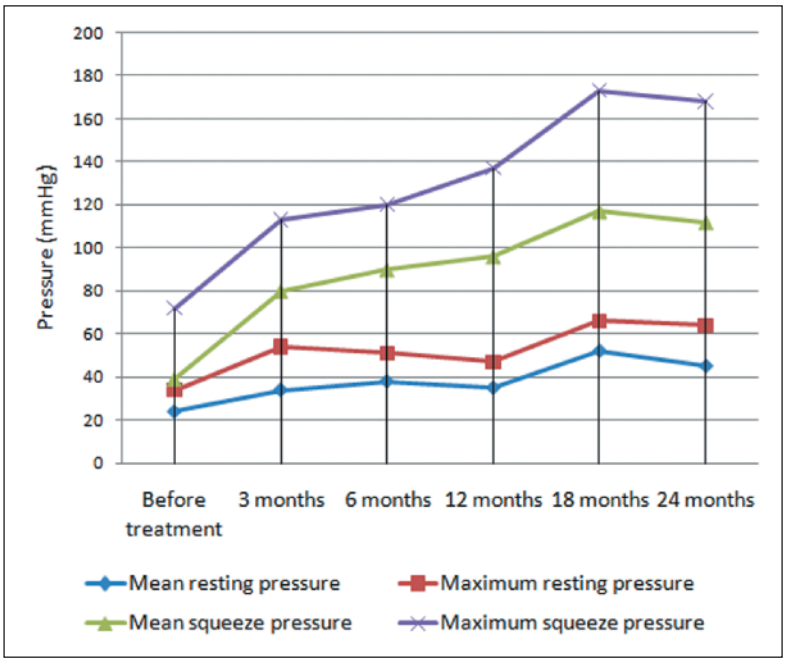


Figure 5. Trend of the Wexner Incontinence Score. Trend of the Wexner Incontinence Score (0-20) for each patient before and at 3, 6, 12, 18, 24 months after Lipogems treatment. * $p<0.05$.

Figure 6. Mean and maximal anal squeeze and resting pressure. Change in mean and maximal anal squeeze and resting pressure scores in 5 patients before and 3, 6, 12, 18, 24 months after Lipogems treatment.



from 6 months up to 24 months was clearly observed at palpation a progressive increase in the contractile activity of the anal sphincters. Furthermore, after 3 months it was appreciated in *patient C* and *E* the presence of a moderate anocutaneous reflex not present prior to the treatment, while in *patient B* was evidenced a more active reflex.

Ultrasound findings

During intraoperative Lipogems® treatment, respect to preopretaive endoanal ultrasonography (Figure 8a), the deposits of Lipogems® were identifiable by the ultrasound scan as hyperechoic spots (Figure 8b).

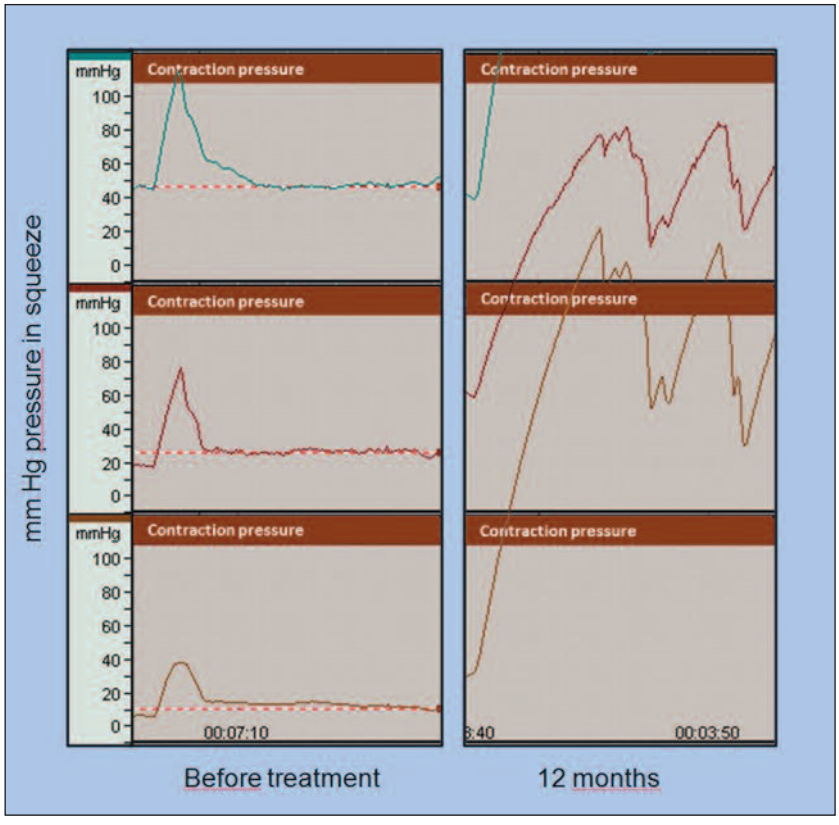


Figure 7. Manometric curves. Patient A: manometric curves in squeeze recorded before and 12 months after Lipogems treatment.

Three months after Lipogems® treatment, endoanal ultrasonography evidenced only an initial resorption of the depots of Lipogems with persistence of widespread hyperechoic spots at sites of inoculation (Figure 8c). From 6 to 12 months ultrasonography detected a progressive resorption of the hyperechoic spots of Lipogems tissue product (Figure 8d). At the same time, endoanal ultrasound identified increasing of muscle fibers at the site of previous injury and a recovery of the circle of the anal sphincters still evident at 24 months when deposits of Lipogems tissue were no longer evident (Figure 8e, 8f).

Discussion

As a result of preliminary reports of efficacy and safety in different medical specialties, regenerative therapy with the use of autologous stem cells, freshly harvested or culture-expanded, recently has been used for treating those conditions that usually are unmet and result in poor outcomes or invasive surgery[58]. In recent years, autologous stem cells have been used for experimental treatment of patients with urinary incontinence and anal incontinence. In these studies, bone marrow and muscular tissue constitutes the most frequent source of adult MSCs[35,60-62], although this cell population is quite rare in these tissues (0.001%-0.01%)[63]. Recently, the human adipose tissue has been identified as a convenient source of MSCs, generically referred to as "adipose-derived" stem cells (ADSCs)[64]. In this study we report, for the first time, the use of a fat tissue regenerative product (Lipogems®) obtained with a closed, non-enzymatic method[37,39], to treat patients with anal incontinence. Other *in vitro* observations indicate that MSCs are encapsulated in the fat cell preparation and are the active agents of the Lipogems® procedure.

Lipogems tissue appears as a fat grafting procedure but the fat tissue which is injected, compared to standard lipoaspirate, is composed of very small clusters with viable and intact pericytes and MSCs in their own natural niche[36,37]. The particular high

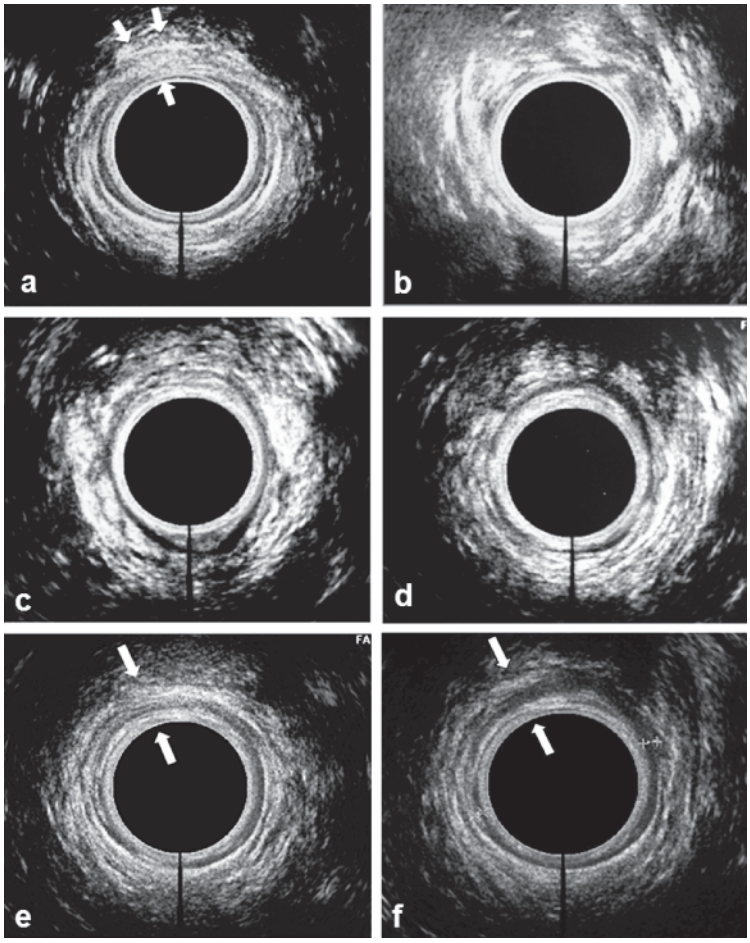


Figure 8. Endoanal ultrasonography. Endoanal ultrasonography images of a representative patient, Patient A, at preoperative, intraoperative stage and at 3, 6, 12, 24 months after treatment. *A*, Preoperative endoanal ultrasonography*. Lesion of the external and internal anal sphincter localized in the anterior area of the muscular rings; *B*, Intraoperative ultrasonography*. Diffuse hyperechoic spots at the sites of inoculation of Lipogems; *C*, Ultrasonography* at the 3rd month. Partial resorption of hyperechoic spots of Lipogems; *D*, Ultrasonography* at the 6th month. Appearance of initial development of new muscle fibers at the site of the lesions. Are still evident few hyperechoic spots of Lipogems; *E*, and *F*, Ultrasonography*, respectively, at the 12th and 24th month. The image show a kind of muscle repair. No more evidence of hyperechoic depots of Lipogems *(scanned at mid anal canal level).

concentration of stromal-vascular fraction present in clusters of Lipogems® tissue, after the elimination of most of the fat cells during the processing in the device Lipogems®, is also detectable by the characteristics of the ultrasound image achieved with endoanal ultrasonography. In fact, at the ultrasound scan, the areas of inoculation of Lipogems® product appear hyperechoic, indicative for high concentration of hyperdense cells (Figure 8b), whereas, the adipose tissue has a lower density which gives hypoechoic ultrasound images[65,66].

The technique of injection into the patients has proven to minimally traumatic and very smooth due to the fluid nature of Lipogems® which can easily pass through fine sharp or blunt needles (21 up to 25 gouge) and can uniformly be distributed in target tissues. The method of distribution of the microfragmented fat graft that we employed (Figures 2, 8), more than obtaining a favorable bulking effect on the anal canal, provides substance able to repair lesions of the anal sphincter apparatus related to muscle and nerve. Moreover, as fecal incontinence causes considerable apprehension, we used a validated instrument to address personal implications in patient quality of life. We demonstrated that after Lipogems® treatment, the FIQL score for all patients increased from preoperative condition.

Indeed, regarding the FIQL score described by Rockwood on psychometric evaluation, based on four scale (Lifestyle, Coping and behavior, Depression/self-perception, Embarassment) in patients affected by fecal incontinence and in subjects known not to have Fecal Incontinence, but other gastrointestinal problems (control group), our patients, after 3 months of treatment and for all the follow-up period, showed the same value (3<FIQL<4) to the control population[57].

By a comparison of patients data, in our experience, we observed a slight worsening of symptoms of incontinence simultaneous to a moderate decrease of resting pressure in the interval between the 3rd month and 12th month (Figures 5, 6). In this same period of time, endoanal ultrasound revealed that the deposits of inoculated Lipogems®, were gradually reabsorbing, as evident in Figure 8c, 8d. Later, between the 12th and the 24 month, there has been a stabilization of the symptoms of incontinence, while there was a small increase in pressure at rest associated with an evident increase in mean squeeze pressure and maximum squeeze pressure. During the second year of follow-up, after the absorption of the cellular component of adipose tissue, the ultrasound image showed repair of the anal sphincter with restitutio ad integrum of the muscle fibers (Figure 8e). The slight worsening of the symptoms of incontinence between the 3rd and 12th month is, in our opinion, attributed to absorption of the adipose component of Lipogems® with loss of bulking effect which usually occurs after 6 months to 1 year as is also observed with treatments employing the traditional technique of lipofilling which always require multiple filling[41,15,67].

An attractive feature was the clear increase in the contractile ability of the sphincters recorded by anorectal manometry after 12 months (Figures 6, 7) when deposits of material inoculated disappeared and ultrasound images showed muscle repair. Frudinger et al[35] reported the use of autologous myoblast cells cultured from a biopsy of pectoralis muscle injected into anal sphincter defect to treat patients with anal incontinence due to obstetric injury. They report only a transitory increasing of the maximum anal squeeze pressure at the 1 and 6 month assessments, returned to baseline at 12 months after treatment. While Frudinger et al have used muscle-derived cells prepared by enzymatic/chemical procedure and cultivated, we used a tissue graft prepared mechanically and in the same surgical session.

The Lipogems® tissue graft preserves the tissue micro-structure with intact niche that allows a functional "regenerative unit". In this way, the MSCs and pericytes contained in the niche are probably both more available and functionally intact than isolated injected cells to promote tissue regeneration[58]. In our initial experience, it is possible that the activation of MSCs has produced muscle repair? This suggestion is supported by the impressions of other authors[68] and there are also studies *in vivo* and *in vitro* on the capacity of the MSCs to produce regeneration of musculoskeletal cells. In a recent study, Lorenzi[31] described treatment of anal sphincter injury artificially induced in rats with injection of BM-MSCs. In addition to the functional results, he evaluated histological examinations performed on animals sacrificed. At the site of injury, he described an area rich in irregularly placed muscle cells of different sizes that indicated the presence of selective regeneration of anal sphincters. In a recently article, Kajbafzadeh[69] has investigated the inoculation of autologous muscle progenitor cells in rabbits previously submitted to external anal sphincterotomy. In addition to an enhanced anal contractility evident to manometry, the histomorphological studies of damaged external anal sphincter detected a significant decrease in interstitial fibrosis associated to regen-

erating muscle fibers with a variable orientation, significant for new muscle fiber regeneration.

The improvement in contractility and anal incontinence symptoms recorded in *patient B* and *E,* but especially in *patient C,* are striking with regard to the improvement of peripheral nerve function. The mechanism for this beneficial effect is not clear and several kinds of mechanisms, such as stimulating neovascularization, immunomodulation, and neurotrophic influences, may be hypothesized. However, in the literature there are only experimental studies on the regeneration of peripheral nerves with use of MSCs and there is no sure clinical evidence of peripheral nerve regrowth after regenerative MSCs therapy[25-28,70-74]. This single observation is certainly very suggestive, but it is to be verified with further and more detailed investigations.

Conclusions

This new technique seems to give remarkable clinical results with a very simple regenerative procedure and minimally traumatic surgery which is very well tolerated by patients.

Declaration of interests

Carlo Tremolada is the inventor of intellectual property used in this study and an equity owner in Lipogems International, SpA, licensee of the patents used in this study.

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