Title of the project:

Quick solution for bone fusion disorders

Project Team

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Project goal (idea) and end result (one short paragraph)

Idea:

Bone fusion disorders require bone reconstruction, as they can lead to further complications. Non-unions are the most common complication after bone fractures in both humans and small animals – the latter due to their anatomical conditions. The main challenge is the surgical management of non-unions, since besides bone stabilization, the fracture site must be provided with adequate osteoinductive scaffolds and/or growth factors and a supply of osteoblastic cells that will form a new bone. We provide a solution to this problem thanks to the invented method of rapid and efficient homing of adipose tissue derived cells to osteoblastic cells. The stromal vascular fraction cells harvested from the subcutaneous adipose tissue can be quickly induced *in vitro* to form osteoblastic cells, followed by their local administration to the bone fracture – either on the scaffold used in this invention or any other suitable surgical material. The presented solution can be applied in orthopedics and veterinary medicine.

Key words: non-unions treatment; bone restoration; subcutaneous adipose tissue; autologous transplantation; osteoblastic cells

State why the idea is unique, innovative or revolutionary?

The presented solution offers several advantages over limited existing methods. Supporting bone fusion with platelet-rich plasma (PRP) is inefficient; implantation of available biomaterials without a biological component (i.e. growth factors or cells) is insufficient to initiate bone fusion. Our protocol allows for **atraumatic** harvesting of cells that can be **rapidly** prompted to osteoblastic phenotype capable to form a new bone, followed by their delivery to the non-union site. The laboratory procedures are **simple** and **easy** to set up in a clinical setting, the **short time** gap between adipose tissue cells' harvest and osteoblastic cells delivery can be as short as 3 days for autologous cells transplantation and potentially, the protocol can be shortened and bypass a pre-surgery visit to harvest adipose tissue cells in case of allogenic transplantation. Eventually, the revived implant can be delivered to the non-union site to provide both a scaffold for missing bone and osteoblastic cells that support new bone formation. In a sharp contrast to standard differentiation methods of adipose tissue cells to bone-like cells, taking up to 4 weeks in culture, our method can provide osteoblastic cells much faster, within 3-7 days.

Project description

Subject matter description

Incidence of bone fracture is very common in both medical and veterinary profession. Despite tremendous progress in medical and veterinary fields to address this problem, the complication rate in the treatment of bone fractures remains relatively high, i.e. 28% according to Andrade et al. 2025. Bone fusion disorders are a very serious problem in orthopedics and traumatology. The surgical treatment of non-unions, which account for 16% of cases after bone fractures, still presents a challenge. This is due to the needs of stabilizing the bone along with providing the site with osteoinductive scaffolds and/or growth factors, and a supply of osteoblastic cells that will form a new bone.

Autologous transplantation of spongy bone is currently most commonly used for this purpose, despite it prolongs the operation time and causes additional trauma. For small animals, especially dogs of miniature "toy" breeds with a body weight < 1 kg, this type of procedure is very difficult. The growing popularity of these breeds significantly increases the owners' needs for solving the problem of e.g. forearm bone fractures in these animals. They are particularly acute due to anatomical conditions (i.e. congenital disorders of vascularization of the forearm bone and significantly reduced intraosseous blood supply to the distal part of the radius) making the complication rate **up to 75%!** There are currently no effective methods of supporting the aforementioned fractures with biological methods due

to the animal size and the potent trauma accompanying, e.g., bone marrow biopsy in an animal weighted < 1 kg.
The persistent state of bone non-union both in humans and animals eventually leads to bone atrophy and permanent deformity of the limb and frequently to the need for amputation.
End result: We offer a quick solution to this problem by using the adipose tissue-derived stromal vascular fraction cells, harvested from subcutaneous adipose tissue, that in our innovative protocol, can be rapidly and effectively prompted to provide osteoblastic cells, followed by their delivery to the bone fracture site on the scaffold used in this invention or any other suitable surgical material. It should be noted that the scaffold proposed in the invention is a combination of synthetic/autologous material that does not carry the risk of transmission of infectious diseases.
The proposed solution is based on the invented protocol of rapid differentiation of adipose tissue cells into bone-like cells *in vitro*. The protocol includes bioactive material as a cell carrier; the developed composition of culture medium supplements ("chemical cocktail"), and application of dynamic cell culture conditions.

Current status and key findings

The proposed solution is based on quick and efficient guiding of adipose tissue-derived cells to differentiate into bone cells *in vitro*, thanks to the application of: specific bioactive growth surfaces; a defined chemical cocktail to stimulate cells in culture; and specific dynamic culture conditions. The method has been verified *in vitro* in several human and animal cell types and we expect to verify it in animal models in near future, preferably mammals other than rodents. Thus, at present we declare TRL IV technology readiness level.

Ultimate goal

The presented technology has tremendous potential to improve the treatment of bone fractures in orthopedics and veterinary medicine (especially miniature dog breeds). The latter is of special interest, given that neither method described so far in the scientific literature as to stimulating the bone fusion process in small dogs, has not entered clinical practice on a massive scale. Giving the above-mentioned animal model studies are completed, it will be possible to request clinical trials and subsequent implementation of the discovered therapeutic agent in domestic animals, starting with the treatment of miniature dog breeds, followed by the potential adjustment of the developed product to treat humans.

It should be highlighted that the application of the Innovation in humans opens up completely new treatment options in the fields of orthopedics and traumatology, such as: 1) treatment of fusion disorders especially of the atrophic type, requiring not only improvement of stabilization of bone fragments, but also boosting local bone formation; 2) restoration of bone defects in (a) complex open fractures requiring removal of devitalized bone fragments, especially with significant contamination of open fractures, due to additional antimicrobial effects of applied cells; (b) intra-articular fractures; (c) after removal of benign tumor lesions; (d) after clearing the focus of necrosis upon sterile ischemic necrosis of the femoral head; (e) upon loosening of joint (especially hip) endoprostheses. Innovation can also accelerate the remodeling of the applied bone graft materials and the osteointegration of revision implants. Besides, the antimicrobial effects of applied cells can reduce the risk of infections, the percentage of which in revision procedures can reach 22%, whereas the filling of bone defects during revision procedures in sports medicine can serve to fill bone channels during two-stage revision ligament reconstructions of the knee joint.