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RESEARCH ARTICLE

EVALUATION OF THE EFFECT OF FREE OMENTAL TRANSPLANT ON EXPERIMENTALLY INDUCED BONE DEFECTS OF WEST AFRICAN DWARF GOATS

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Abstract

The effect of free omental transplant on experimentally induced bone defects was studied in West African dwarf (WAD) goats. Three WAD goats (all male) of six months of age were used for the experiment. A-four millimeter diameter bone defects were aseptically created under anaesthesia on the medial aspect of the left and the right tibia of each of the animals following a standard procedure. The right tibia of each goat was implanted with free autologous omental flap while the left tibia of each goat had no implant and served as the control. The omentectomy was done following standard procedure. The soft tissue wounds were closed routinely. The post operative radiographs of the bone defects were taken at weeks 0, 2, 6 and 10. The level of callus proliferation, mineralization and maturation of the osteoid at and around the defects were evaluated and compared in the 2 groups. The result demonstrated that the right tibias which had the omental flap transplant had superior osteogenic activity in terms of early bridging callus of greater sclerosis and also earlier incorporation of mineralized callus into the adjacent cortices which meant early remodeling.

INTRODUCTION

Researches into the biology of bone, ligament and tendon healing have led to the development of variety of products designed to help stimulate biologic factors and promote healing (Foster *et al.*, 2009). The use of exogenous recombinant proteins including bone morphogenic proteins is being investigated. Application of mechanical vibration along the axis of the fractures, ionic resonance electromagnetic field stimulation, and static magnetic force with Samarian cobalt magnets has all been employed. In our rural setting, many herbs have been investigated with varying degree of successes and failures. Previous studies on the repair of segmental defects have focused on bone matrix substitutes (Grundel *et al.*, 1991; Delloye *et al.*, 1992; Gogolewski *et al.*, 2000). However, these substitute matrices do not perform as well as autograft for several reasons including histochemical responses by the host tissue and a dearth of living cells. Recently, the osteogenic potential of concurrent application of autologous cancellous bone grafts and autologous platelet-rich plasma on experimentally induced ulna defects of dogs has been elucidated (Nnaji and Kene, 2015; Nnaji *et al*., 2015).

Although remarkable progress has been made in enhancing osteogenesis of different bone defect models, cost and availability of some of the biomaterials used as osteogenic adjutants makes their use almost impossible especially in our rural settings and in most underdeveloped countries of the world. This goes to buttress the rampant cases of delayed and non union fractures that still abound in many orthopedic clinics around the world.

In view of this predicament, developing a cheap, easily available and cost effective biodegradable material that can promote bone healing, without histochemical incompatibility becomes very much imperative. One such biological product that is been vigorously investigated by many researchers around the world is the omentum.

The omentum is a highly vascular, fatty tissue, approximately 14-inches long and 10-inches wide which hangs like an apron over the intestines and lower abdominal area of goats. Although the omentum had been viewed as an inert tissue bereft of significant biological function, scientists are now discovering that it is an intriguing, physiologically dynamic tissue with a considerable body of research that supports its therapeutic potential (Goldsmith, 2000; Agner *et al*, 200). It has been established that the omentum contains angiogenic factors that stimulate the growth of new blood vessels into whatever tissue it is surgically placed next to, including the brain and spinal cord **(**Bader 2011; 10Karimi *et al.*, 2013). It is rich in lymphatic vessels and tissue that are critical in removing metabolic waste and excess fluid, destroying toxic substances, and fighting diseases. The omentum has also been found to be a rich source of biological material that enhances tissue growth, including angiogenic factors, key neurotransmitters, nerve growth factors, and agents involved in inflammatory and immune processes (Topor *et al*, 2001). Evidence suggests that omental tissue contains stem cells which are pluripotent master cells that can differentiate into a variety of cell types (Neurological Research, 2005). These cells were shown to synthesize key growth factors that promote vascularization when transplanted.

With all these pluripotent properties inherent on omentum, it is most likely that it may be of serious osteogenic values in management of bone defects in many animals. This study was therefore designed to evaluate the osteogenic effects of free omental transplantation on experimentally induced tibia defect model of West African dwarf goat.

Materials and methods

Ethics statement

This work was carried out in accordance with the guidelines for animal experiments released by the National Institute of Animal Health. This study was approved by the Animal Welfare Committee of the Faculty of Veterinary Medicine University of Nigeria, Nsukka.

Experimental animal

Three goats of 5 months of age were bought from a household at Nsukka in Enugu state, southeastern Nigeria and they were left to acclimatize for three weeks before being subjected to the study. They were housed in standard animal house, fed on quality pastures, supplemented with concentrates. They had access to water ad libitum and the surgery was conducted under strict aseptic conditions. In all the animals, the right tibias were designated as the experimental group while the left tibias served as control.

Omentectomy

Following careful clinical and physical examinations to certify the health status of the animals, each was taken into the preparatory room for a generous shaving on their left flank and the medial aspect of the thigh using razor blades, soap and water. The shaved areas were aseptically scrubbed with hibitane impregnated gauze sponges. Then 0.1mg/kg of xylazine hydrochloride was administered intramuscularly followed by an inverted L-block of the left flank with 2% lignocaine hydrochloride. Each goat was then draped with sterile shrouds ready for the surgery.

An incision of about 3-5cm long was made on the left lower flank into the abdomen, cutting through the skin, the subcutaneous fascia, deep fascia, external abdominal oblique muscle, rectus abdominis muscle, transverse fascia and the parietal peritoneum-which forms the inner lining of the abdominal cavity. The incision of the parietal peritoneum exposed the rumen on whose surface the greater omentum was located. A section of the greater omentum (1cm in diameter) was excised and placed in an isotonic solution. Using size 2/0 chromic catgut, the peritoneum and the muscle layers were suture in a simple continuous pattern. Subcuticular stitches were also applied to appose the subcutaneous tissues. The skin was finally suture with size 2/0 nylon suture in a simple interrupted suture pattern.

Osteotomy and omental impantation

Another skin incision of about 3cm was made on the medial aspect of the right tibia of each animal. The muscles of the long digital extensor, lateral digital extensor, and deep digital flexor were located and carefully retracted to expose the tibia. Care was taken not to rupture the tibial artery and the peroneal nerve which run along the tibia bone. With a bone drill and a drill bit, a 4mm diameter circular defect was made on the tibia. Haemostasis was achieved using hemostatic forceps and gauze sponges. The excised omentum was then implanted on the bone defect. The muscles were then apposed together to cover the bone with the implant using size 2/0 chromic catgut in a continuous pattern. The subcutaneous tissues were apposed with subcuticular stitches. The skin incision was finally closed with size 2/0 nylon in a simple interrupted pattern. This procedure was repeated on the left tibia but without omental implantation on the defect to serve as the control.

Post operative care

The physiologic parameters were monitored for three days post treatment while watching for any complications. The skin wounds were dressed daily for ten days after which the stitches were removed.

Radiographic evaluation

Radiographs of the left and right tibias were taken for each of the WAD goats at weeks 0, 2, 4 and 8 post-implantation. At the end of the 8th week, the animals were euthanized and the affected tibia bones (left and right) dissected out and fixed in buffered formalin. The cut segments were decalcified, embedded in paraffin wax, sectioned onto slides and stained with Hematoxyline and Eosin (H & E). The slides were later evaluated and compared for histological changes.

Results and Discussion

The clinical evaluation of the two groups revealed soft tissue swelling at the sites of operation. The swelling on the defects with omental transplant was however larger than those of the control. This could be due to increased thickness of the defective area as a result of the added thickness of the implanted omentum or it could be ascribed to increased inflammatory reaction at the sites elicited by the bioactive implants (Kalfas, 2001; Hishida, *et al*, 2010). The radiological results of the two groups revealed that the treated group demonstrated superior osteogenic activity which is summarized in Table (1).

Table 1

Week	Control group	Treated group
1	Radiographs showed clear outline of the defects with no evidence of endosteal and periosteal reactions at and around the sites (Fig.1b).	Radiographs showed clear outline of the defects with no evidence of endosteal and periosteal reactions at and around the sites (Fig.1a).
2.	The edges of the circular defects are still sharply outlined with no radiologically visible evidence of endosteal and periosteal callus deposition at and around the defects (Fig.2b).	There is dense periosteal reaction around the circular defects which slightly obliterate the marrow cavities at the edges of the defects (arrow) (Fig.2a).
6.	Minimal external callus formation which was of soft tissue density around the defects is evident, but the circular outline is still clearly visible (Fig.3b).	Dense external callus formation bridges the defects, making the outline of the defects invisible (Fig.3a).
10.	Large external callus formation around the defects with no periosteal incorporation (Fig.4b).	Small size of external callus around the defective sites following incorporation of the callus into the adjacent cortical bone. Note the higher degree of sclerosis of the adjacent corticies and the uniformity of the osteoid density of the marrow even at the site of the defects (fig.4a).

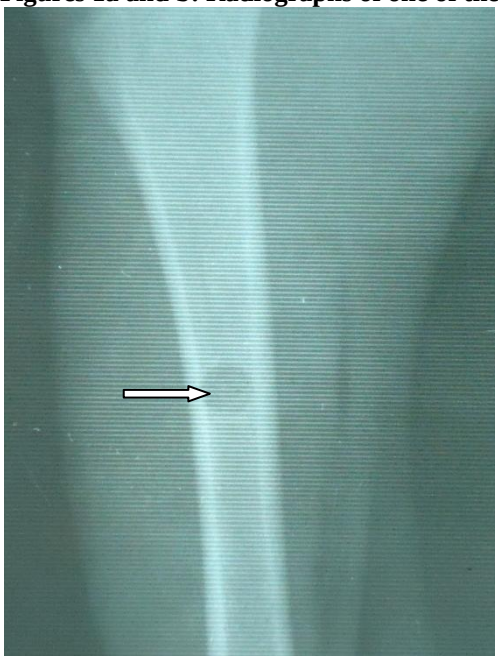
At week 2, no visible radiologic evidence of endosteal and periosteal callus deposition at and around the control defects were seen but in the treated group, slight osteogenic reaction at the same time was observed. At week 6 there was little endosteal soft tissue callus formation among the control, but this was not comparable to those of the treated animals where osteo-dense endosteal and periosteal callus were seen. This could be due to the presence of omental flap, which helped in the revascularization of the defects. It has been documented that the omentum could be a good vascular bed that promotes angiogenic activity and healing of tissues in which it is in contact with (Kobayashi, 2000; Maloney, 2004; Alagumuthu, 2006). The minimal external callus formation which was of soft tissue density seen in the control defects coupled with the clearly visible outline of the defects as compared to the dense external callus which bridged the defects among the treated group, could be due to the periosteal proliferation which played an important role in formation of compact bone in the treated group. The greater density of the osteoid at week six in the treated defects signified increased bone mass as a result of the formation of hard callus following mineralization (Nnaji and Kene, 2015).This was possible since omental adipo-cytes, are primary source of vascular endothelial growth factor (VEGF protein), which can stimulate bone repair not only by promoting angiogenesis but

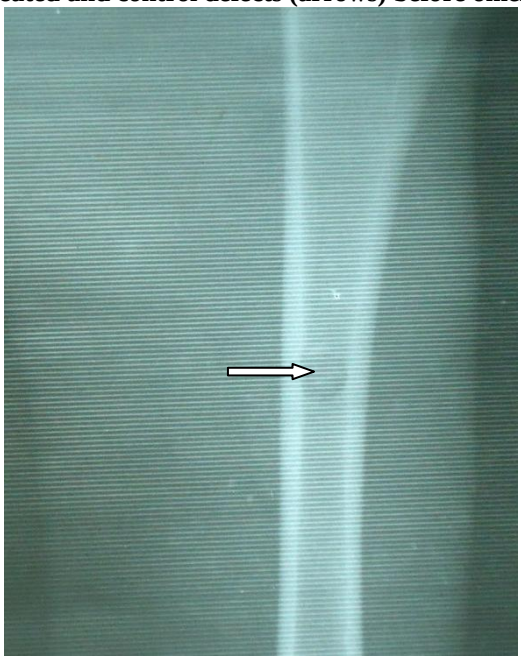
also by accelerating and enhancing bone turnover (Mahdi, 2005). Also, VEGF directly promotes the differentiation of primary osteoblasts and play an important role in callus formation. Normally, the repair of musculoskeletal tissues generally starts with the formation of blood clots and degranulation of platelets, which releases growth factors and cytokines at the sites. This microenvironment results in chemotaxis of inflammatory cells as well as the activation and proliferation of local progenitor cells (Tabata, 2003). Enriching this microenvironment with exogeneous growth factors and other cytokines inherent on omentum could have contributed immensely to the greater osteogenic activities and enhanced healing of the defects seen in the treated group (Nnaji and Kene, 2015). At week 10, the treated defects had undergone appreciable remodeling with clear evidence of periosteal incorporation into the cortex at the site of the defects. The callus density at the defect was equal to the adjacent cortex density. For the control, extensive callus proliferation was still on, with massive periosteal callus covering the defects. The callus density was not yet equal to the adjacent cortex density which signified poor mineralization, lesser bone mass and poor osteiod density as compared to the treated group. Presence of abundant blood vessels in omentum is a good source of nutrients, oxygen, angiogenic and growth factors, and cold have created a proper microenvironment for tissue induction (Glowacki, 1998; Moore, 2002).

Appropriate vascular flow from the omentum could have increased the oxygen concentrations, and resulted in production of the osteoprogenitor cells from the perivascular mesenchymal cells (Diaz-Flores, *et al.*, 1992; Glowacki, 1998; 22).

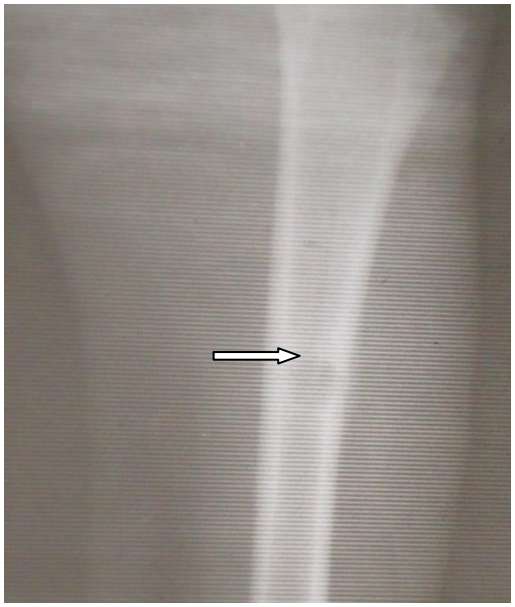
In conclusion, this study has demonstrated that omental pedicel flap could play an important role in enhancing bone healing of West African dwarf goats through the acceleration of osteogenesis.

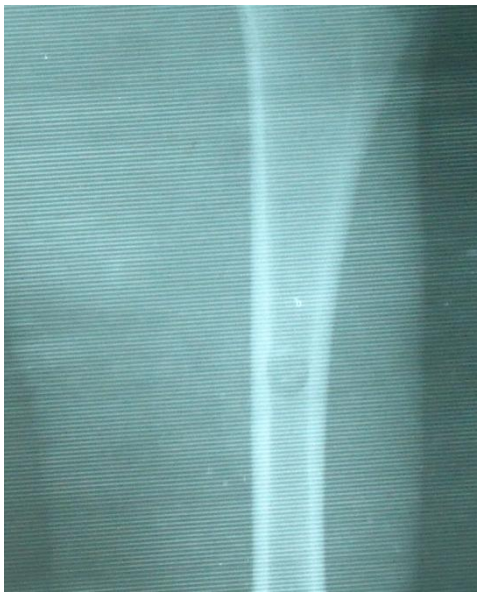
Figures 1a and b: Radiographs of one of the treated and control defects (arrows) before omental implantation


1a


1b

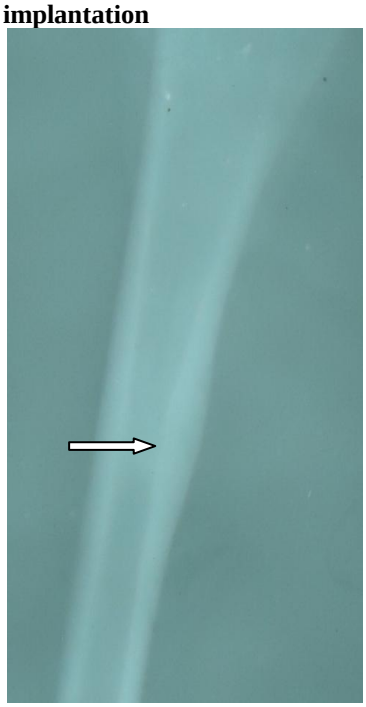
Figures 2a and b: Radiographs of one of the treated and control defects (arrows) at week 2 post omental implantation


2a


2b

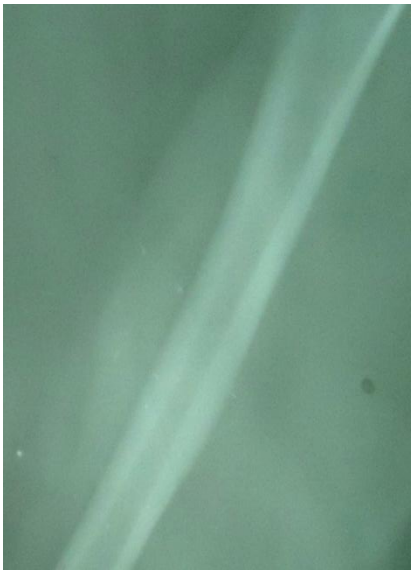
Note the greater osteiod density around the periphery of treated defect (arrow) as compared to the control.

Figures 3a and b: radiographs of one of the treated and control defects (arrows) at week 6 post omental implantation

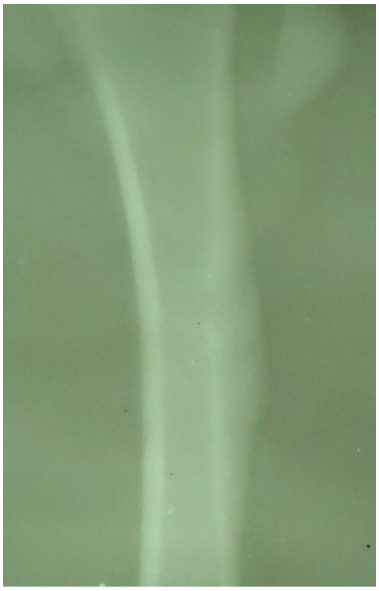

3a


3b

Figures 4a and b:Radiographs of one of the treated and control defects (arrows) at week 10 post omental implantation



4a



4b

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