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DELO

Tissue Engineering in Slovenia Treatment with Cultured Cartilage Cells and Bone Tissue



Dr. Nevenka Kregar Velikonja, Managing Director of Educell Ltd.

Today, when certain procedures such as obtaining and implanting autologous chondrocytes, in other words, treatment of injured joint cartilage using the patient's own cells cultured in the laboratory, have long since become routine clinical practice, we need simply to recall the group of expert enthusiasts who with vision, expertise and enormous energy take the credit for setting the foundations of today's successful image of Educell: consultant Damjan Radosavljevič, an orthopedic specialist, brought the idea of cartilage tissue engineering to Slovenia and put the method into clinical practice; Asst. Prof. Dr. Matjaž Jeras started to develop the technology of preparing tissue engineering products in the laboratory; Asst. Prof.

Three-dimensional cartilaginous replacements for simpler operating procedures in treating cartilage injuries, bone replacements for treating injuries to the jawbone, inserts of autologous cartilaginous cells to prevent contraction of the intervertebral space and the use of autologous cartilaginous cells to treat vesicoureteral reflux, which is a major cause of chronic kidney infection. These are the main development projects, some of which have also successfully been implemented into clinical practice, recounts Dr. Nevenka Kregar Velikonja, managing director of Educell, the cell biology company.

As a biologist, our interviewee devoted her master's studies to human genetics, and in her doctoral work she focused on biotechnology, and she falls into the group of experts who a little under a decade and a half ago in Slovenia – among the first in the world – took pioneering steps in the area of tissue engineering, or regenerative medicine. The basic activity of the company Educell is the development of tissue engineering methods for the regeneration and treatment of injured or worn out tissue. It operates as a research organisation, and its composition includes a cutting edge research team. The company is owned by the holding Novoline and is also a member of the Ljubljana Technology Park.

Dr. Miomir Knežević saw in this project an entrepreneurial challenge and successfully headed the company in the first five years, while Asst. Prof. Dr. Primož Rožman worked with the team to weave together a vision of developing regenerative medicine. In time

the circle of (external) associates has expanded significantly, and includes research, clinical institutions and academic institutions, while it also extends far beyond the borders, for instance to Columbia University in the USA.

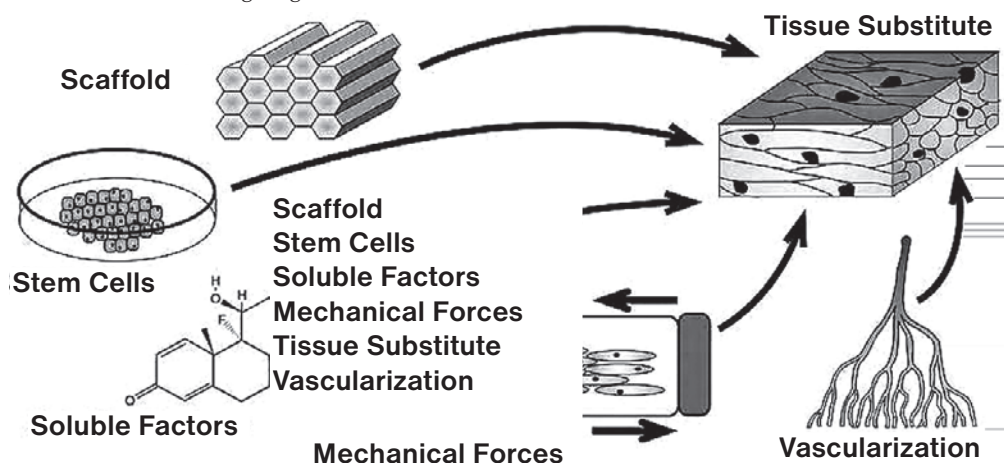


Figure 1: Components of engineered tissue prepared with adult stem cells (Barrilleaux et al., 2006)

Tissue Engineering and Stem Cells

Dr Ariana Barlič, Head of R&D, Educell

Tissue engineering has been defined as “an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function” (Langer and Vacanti, 1993). It consists of culturing cells in an in vivo-like environment that promotes growth, differentiation and 3-dimensional (3D) organisation.

Growth and differentiation factors are added to the culture medium, and cells are encouraged to form 3D tissue within engineered scaffolds of biological or synthetic origin that resemble an extracellular matrix (ECM).

Production of this engineered tissue necessitates the use of large quantities of cells, but this requirement can be difficult to satisfy with



Dr. Miomir Knežević, one of the pioneers of tissue engineering in Slovenia and the first director of company Educell.



Preparation of bone replacement in a protective microbiological chamber, which ensures an aseptic procedure (photo: Hana Krečič Stres).

Educell, Cell Therapy Service Ltd.

Dr Nevenka Kregar Velikonja

The origins of Educell© d.o.o. date back to July 1997, when the company was founded with the goal of introducing cell therapy and tissue engineering to human medicine. In 10 years of our operation we focused on development of tissue engineering methods for repair of damaged tissues such as cartilage and bone that fail biological healing and treatment of pathologic conditions as vesicoureteral reflux. Clinical applications are developed in tight collaborations with clinicians.

Implantation of autologous chondrocytes for treatment of damaged knee cartilage ChondroArt™ is routinely performed in University Clinical centre Ljubljana since 1999. Cell product used for this treatment has undergone development from classical 1D product (cell suspension) to 2D (cells seeded on collagen scaffold) and 3D gel product (cells encapsulated into gelly scaffold, mimicking native environment of cartilage cells), that was recently introduced into clinical practice. The product is being further developed to improve the quality, simplify surgical procedure and cover broader clinical indications.

In this year, also treatment of vesicoureteral reflux with endoscopic implantation of autologous cell product UroArt™, became routine procedure for selected indications. The procedure has been developed in collaboration with UKC Ljubljana, Department of Urology.

Facing limitations of differentiated cells (such as chondrocytes, osteocytes) as cell sources for cell therapies, we started with investigation of different stem cell sources, mainly multipotent mesenchymal stem cells from bone marrow and adipose tissue, but we are searching for alternative sources of multipotent cells. All R&D projects are performed in collaboration with research group of Blood Transfusion centre of Slovenia. We are expert in the cultivation of human cells and routinely perform procedures such as isolation, propagation, freezing, transfection, subcloning and phenotypic characterisation of cultured cells. Cells and tissue constructs are cultured and manipulated in modern, well equipped laboratories considering all necessary standards and guidelines in field of cells and tissues.

Regulatory frame for cell and tissue based therapeutic products is still in progress. In years 2006-2008, EU Directive about the quality and safety of human tissues and cells intended for treatment, was implemented in Slovenian legislation. It also regulates an individual procedure for a custom made products for an individual patient, used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner. At the same time, EC has implemented the Regulation on Advanced therapy medicinal products that will regulate cell and tissue based products and enable patients across Europe to have access to safe and effective therapeutic treatments.

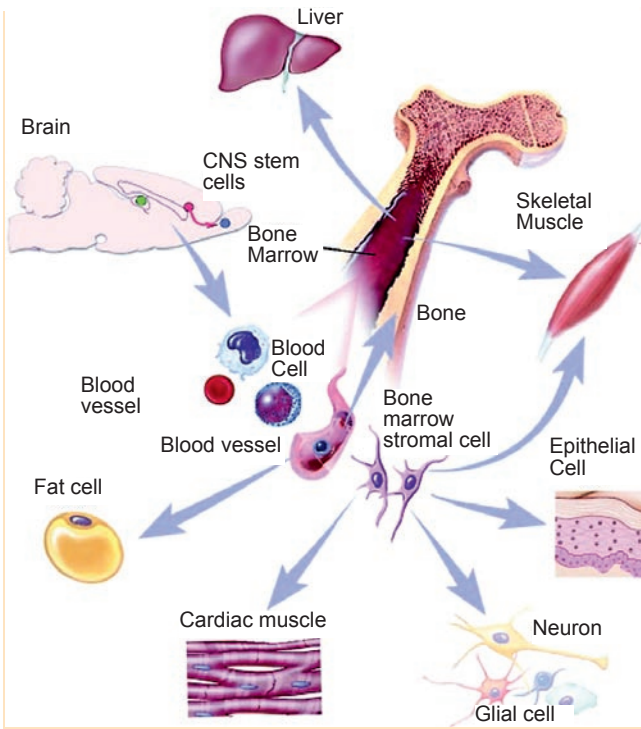


Figure 3: Plasticity of adult stem cells (<http://stemcells.nih.gov/info/basics/>)

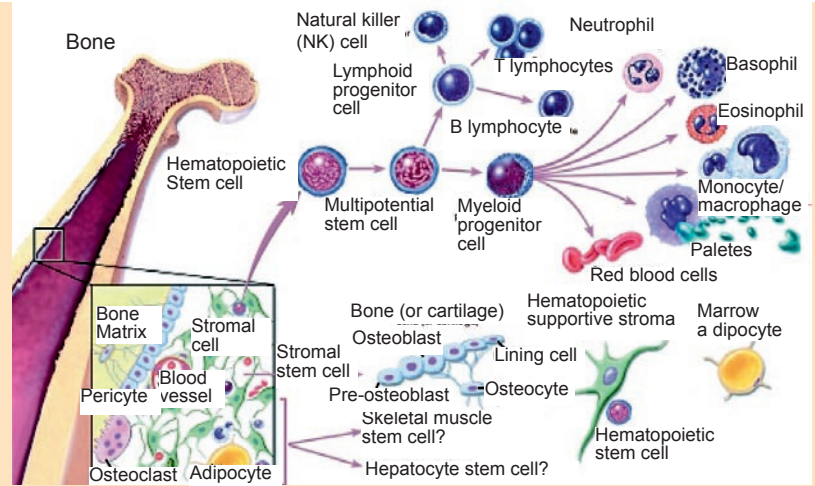
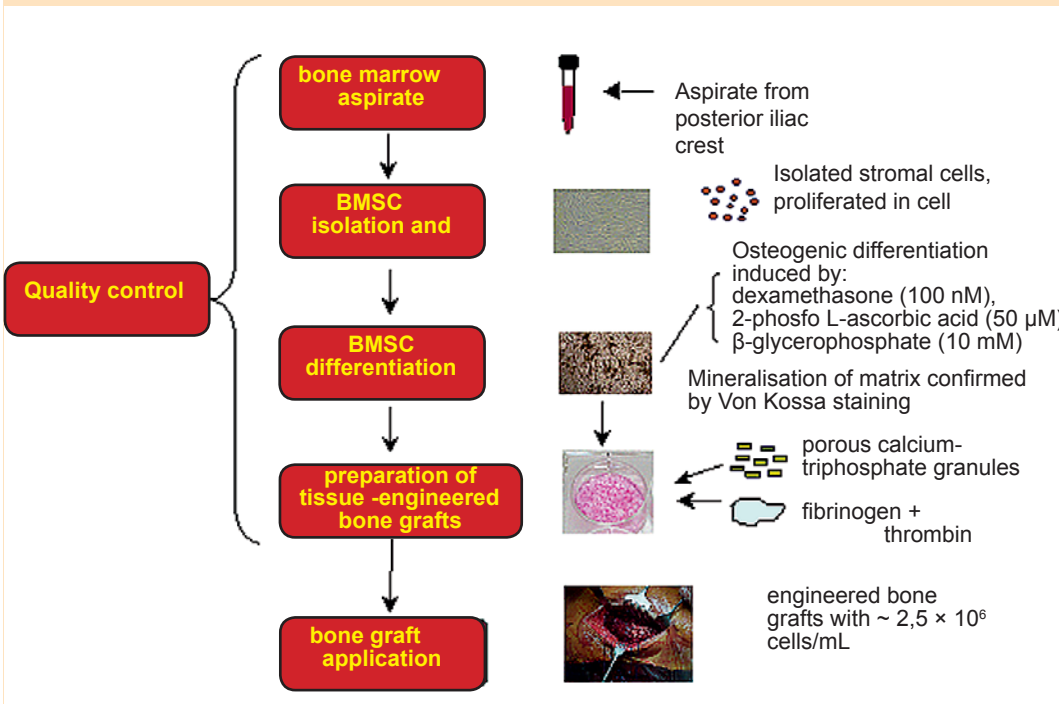


Figure 2: Hematopoietic and stromal stem cell differentiation (<http://stemcells.nih.gov/info/basics/>)

or into cells derived from all three embryonic germ layers (pluripotent). Scientists primarily work with two kinds of stem cells from animals and humans: embryonic stem cells and adult stem cells, which have different functions and characteristics. Both types of stem cells have advantages for therapeutic use; however, problems with teratoma formation and immune rejection must be overcome before pluripotent embryonic stem cells may be applicable for therapeutic use. The benefits of using adult instead of embryonic stem cells to prepare living tissue substitutes include immuno-compatibility of autologous cells, ease of inducing differentiation to a specific lineage and special availability.

Multipotent adult stem cells have been found in many tissues, including bone marrow, adipose, skeletal muscle, cerebral cortex, olfactory bulb core, placenta, gastric epithelium, retina, inner ear, hair follicle bulge, ovary epithelium, testis and others. Aspirates from bone marrow and adipose tissue have been used, because of their mesodermal origin, to engineer such tissues as cartilage, bone, adipose and blood vessels. In addition to cells of mesodermal origin, stem cells from the other two embryonic germ layers, including ectoderm and endoderm, have also been successfully used to engineer tissues.

Under certain circumstances, it would be desirable to harvest autologous stem cells from tissue other than

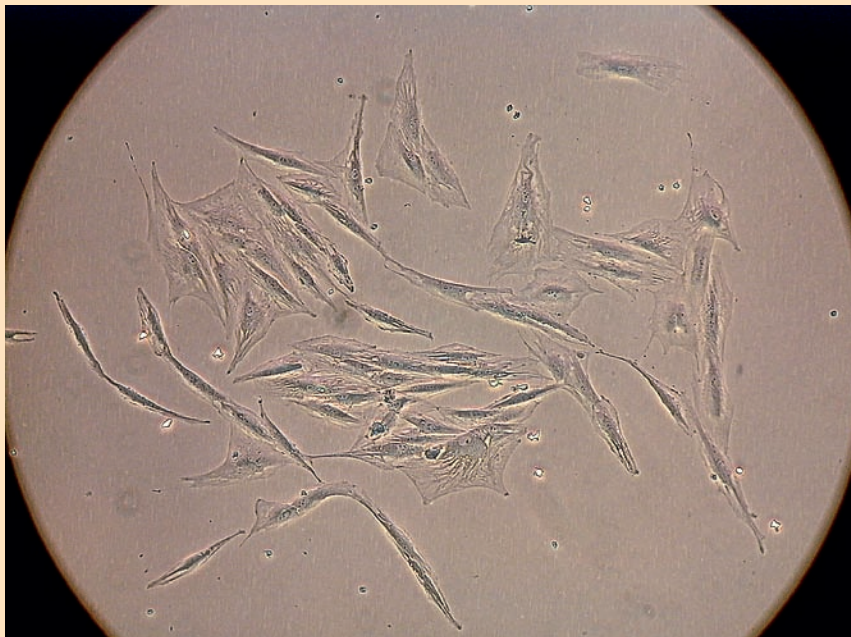


Schematic diagram of tissue engineered bone graft production and implantation (by Hana Krečić Stres, Educell d.o.o.)

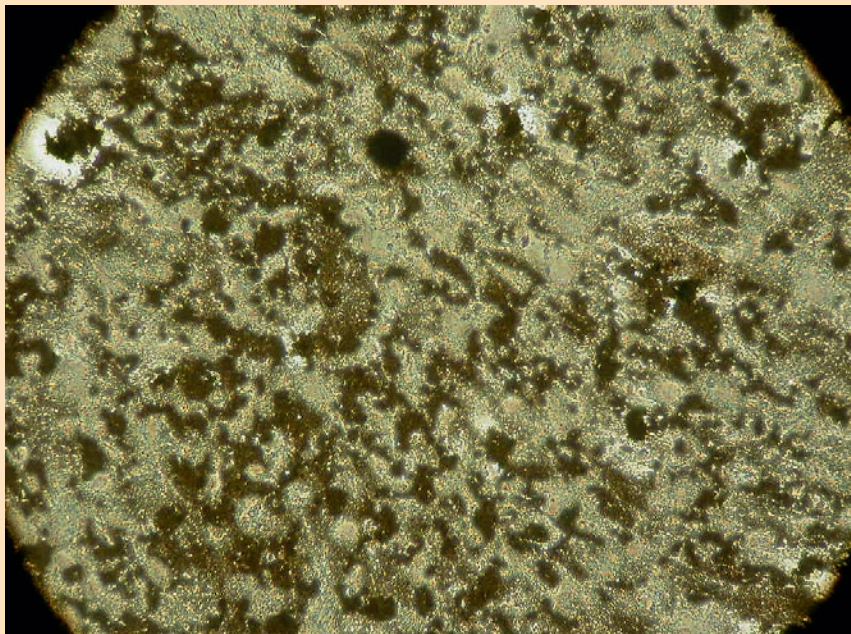
differentiated cells that exhibit limited doublings in culture. One solution to insufficient cell supplies is to prepare engineered tissue with stem cells.

Stem cells have two important characteristics that distinguish them from other types of cells. First, they are

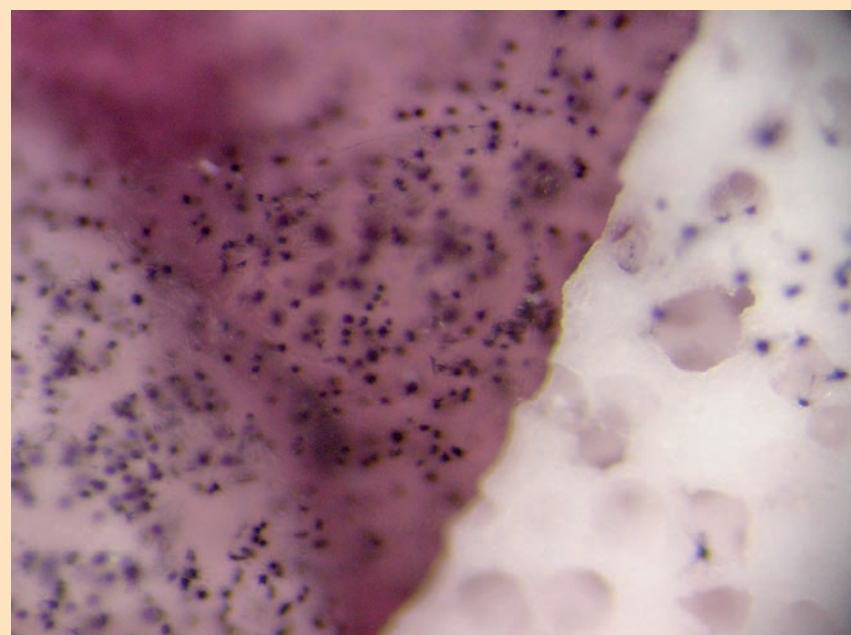
unspecialised cells that renew themselves for long periods through cell division. The second is that, under certain physiological or experimental conditions, they can be induced to become cells with special functions. They have the ability to differentiate along one lineage (unipotent), into multiple cell types (multipotent)



Mesenchymal stem cells in bone marrow create colonies in the cell culture (photo: Nevenka Kregar Velikonja).



Osteogenic differentiation of mesenchymal stem cells appears as an accumulation of mineralised intercellular matter in the cell culture (photo: Hana Krečič Stres).



Testing the survival and metabolic activity of cells in tissue engineered bone replacement with an MTT staining (photo: Hana Krečič Stres).

the targeted tissue to be regenerated, when, for instance, the targeted tissue is diseased or when extraction would cause irreversible trauma. The ability of stem cells to differentiate across the embryonic germ line boundaries is called plasticity. The existence of plasticity has, however, been debated heavily in the past several years (for example, conversion of adult mesenchymal stem cells into cells with a neural phenotype).

Within one research group in Educell d. o. o., we have been working with stem cells from bone marrow for some years. We are developing tissue-engineered bone substitute based on these cells for treating long-bone defects, in collaboration with surgeons of UMC Ljubljana within a project co-financed by the Slovenian Ministry of Defence through the Technology For Security And Peace (TP-MIR) programme. Like other researchers, we are confronting the problems of vascularisation/nutrition of these extensive tissue-engineered constructs. In each step of the procedure from cell isolation to bone graft, quality control measurements are performed.

Facing the limitations of differentiated cells and also bone marrow-derived multipotent stem cells, we started another project (funded through TP-MIR) with the aim of analysing different sources of stem cells in adult human tissues and exploring their differentiation potential. As cell sources, we have chosen bone marrow (in collaboration with the Blood Transfusion Centre of Slovenia), adipose tissue (in collaboration with UMC's Department of Plastic Surgery) and follicular liquid (in collaboration with University Medical Centre's Department of Gynecology). We are analysing differentiation of the isolated and proliferated stem cells into specific cell types (osteocytes, chondrocytes, adipocytes, endothelial cells, neurons, etc.) to evaluate their suitability for different clinical applications.