group, the inter-locking nails were removed at 6 months to provide the repaired femur with a mechanical loading. Results: Radiograph showed that the coral construct was partially degraded at 1 month and completely disappeared at 2 month in control group. Additionally, limited amounts of new bone formed at both ends of the defect were observed from months 3 to 8 post-repair. In experimental group, low radiodensity at the defect area was observed at months 1 and 2. However, radiodensity of the engineered bone continued to increase from months 3 to 6, but remained relatively lower than that of normal cortex bone. At 8 months, the engineered bone was remodeled into cortex bone at the outer layer by the mechanical loading. Difference in radiodensity between two groups is statistically significant ($p < 0.05$). Gross observation at 4 months showed that control defect was filled with fibrous tissue, In contrast, the experimental defect was filled with abundant regenerated bone tissue, which was rich in vascularity. At 8 months, the control bone defect was connected with fibrous tissue and an abnormal movement was observed. However, the experimental defect was completely repaired with tissue-engineered bone, which could bear a strong stress. Histology demonstrated that the control defect contained mainly fibrous tissue in which undegraded coral particle remained visible at 4 months, but completely disappeared at 8 months. In contrast, trabecular bone tissue was observed in experimental defect at 4 months, and irregular osteons was formed at 8 months. Conclusion: Long bone tissue could be engineered in sheep using induced BMSCs and coral. Additionally, coral could be fully degraded within two months, which matches the time period for bone regeneration and thus is an optimal biomaterial for bone engineering.

PP-194
IN VITRO SKIN ENGINEERING USING POLYGLYCOLIC ACIDS

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Introduction: The major concern of using natural matrix for skin engineering is the potential contamination of some currently unknown pathogens. In contrast, biodegradable and bioabsorbable synthetic materials are free of pathogens and easy for quality control. This study intends to engineer full-thickness skin in vitro using polyglycolic acid (PGA). Methods: Human foreskin was harvested and epidermis was separated from dermis by Dispase (0.24u/ml) overnight treatment. The keratinocytes were isolated with trypsin (0.05%)/EDTA(0.53mM) treatment for 15 minutes at 370C. Dermal fibroblasts were isolated by collagenase II (0.1%) digestion for 4 hours at 370C. To test the biocompatibility, both ketatinocytes and fibroblasts were grown on PGA fibers and their growth rates were measured by MTT assay. Cells grown on culture dish served as a control. For skin engineering, fibroblasts were first seeded on a sheet of woven PGA fibers and co-cultured for 1 week in DMEM+10%FBS. The karationcytes were then seeded on the top of fibroblast-PGA construct and cultured in a submerged fashion in serum-free keratinocyte growth medium mixed with DMEM+20%FBS (1:1 ratio). The artificial skin was then further cultured at air-liquid interface in the same medium for another 2 or 3 weeks to form a stratified epidermis. Engineered skin was evaluated with H&E and immunohistochemical staining and electron microscope (EM) at different time points. Results: Both fibroblasts and keratinocytes adhered well to PGA fibers and the former produced abundant extracellular matrices on PGA as demonstrated by scanning EM, indicating good biocompatibility. The fibroblasts were also found to grow faster on PGA fibers than on culture dish. Histology demonstrated that seeded keratinocytes started to form an epidermal structure after 1 week submerged culture, which was composed of single or double cell layers and evenly covered the fibroblast-PGA construct. Active matrix production by implanted fibroblasts was also observed. At 4 weeks, more PGA fibers were degraded than before and a neo-dermis rich in newly formed collagen fibers was formed. On the top, a more matured epidermis was formed, which has stratified into stratum basale, stratum spinosum, and stratum corneum. This maturation was also confirmed by the positive staining of involucrin, a marker of keratinocyte terminal differentiation. Transmission EM also revealed the well-differentiated keratinocytes, which contained spread and fine filaments of keratin in the cytoplasma. Desmosome was observed between the individual keratinocytes. However, hemidesomsome was not observed between the keratinocytes and the basement membrane. Additionally, partial and non-connected structures of basement membrane were observed at this time point, which were also positive for laminin and type VII collagen staining. Conclusion: Double layers and live full-thickness skin can be in vitro engineered with synthetic biomaterial polyglycolic acid. The engineered skin possesses histological structure and biochemical components similar to native skin tissue.

PP-195
HONEYCOMB COLLAGEN SCAFFOLD FOR THE IN VITRO DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO OSTEOBLASTS

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Tissue Engineering based on cell therapy is emerging as a potential alternative to the complicated tissue or organ transplantation.

When cultured in special media with specific growth factors, the mesenchymal stem cells can be differentiated into adipocytes, chondrocytes and osteoblasts.

In the present investigation we have studied the effect of honeycomb collagen scaffold (*Artif. Org. 2001;25:213–217*) for the adhesion, proliferation and differentiation of rat femur bone marrow derived mesenchymal stem cells into osteoblasts.

Mesenchymal stem cells were isolated from about 6 weeks old rat femur bone marrow and cultured in alpha-MEM medium without beta-glycerophosphate and dexamethasone.

Honeycomb collagen discs (15 mm × 1 mm), which was prepared from bovine dermal atelocollagen and cross-linked by heat at 110°C and uv-irradiation, were placed on the proliferating mesenchymal stem cells during the first week of the culture.

The cells were attached quickly on the honeycomb collagen, proliferated and differentiated into osteoblasts.

The differentiated osteoblasts were characterized by measuring the alkaline phosphatase activity and osteocalcin production in the culture.

The osteoblasts also synthesized well-formed calcium crystals in the culture and the same proved by Von Kossa staining and X-ray diffraction analysis.

Light and scanning electron microscopic studies and DNA mea-

surements demonstrated that the differentiated osteoblasts multiplied into several layers on the honeycomb collagen scaffold when compared with control cultures without honeycomb collagen discs.
The results demonstrated that the honeycomb collagen sponge is an excellent scaffold for the proliferation and differentiation of mesenchymal stem cells into osteoblasts.
It also proved that honeycomb collagen is an effective biodegradable substrate for osteoblasts and is very useful in the emerging field of cell based therapies and tissue engineering.

PP-196
ALVEOLAR BONE MINERAL DENSITY AS AN INDICATOR OF SKELETAL OSTEOPOROSIS IN PRE AND POST-MENOPAUSAL WOMEN

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Introduction:
Osteoporosis is a skeletal disorder that frequently results in fractures that lead to pain, deformity, and disability. It is characterized by a reduction in skeletal bone mass and is diagnosed in patients who sustain low trauma fractures, that are associated with substantial costs to the individuals and to the society. Hip fracture rates are projected to double within 15 years and to increase almost fourfold by the year 2041 among elderly white and Asian women. It is anticipated that older adults will constitute an increasingly substantial proportion of individuals needing implant prosthodontic treatment. Unfortunately, wound healing and jaw bone quantity and quality may be compromised by the presence of osteoporosis that attributes to an increased oral implant failure rate.
A correlation was shown between decreased alveolar bone mineral density (BMD), tooth loss and periodontitis. Also, between systemic bone loss and tooth loss due to increased resorption of alveolar bone.
Several techniques have been developed to measure bone mineral density in the axial and appendicular skeleton and in the alveolar bone as well.
Objectives of the present work were:
-To establish a correlation between BMD in the skeleton and BMD in the alveolar bone to help detect people at high fracture risk, in pre and post- menopausal women.
-To study the effectiveness of trabecular bone pattern and BMD in the mandible in detecting skeletal osteopenia and osteoporosis.
Methods:
-Over 150 females above the age of 40 participated in the present work. Each patient was exposed to an osteoporosis risk questionnaire, and oral examination to determine the status of tooth loss and periodontal condition.
-Digital periapical radiographs were performed for each patient using a standardized method to estimate BMD in two directions:
1. Vertically in the interdental alveolar bone between the mandibular posterior teeth.
2. Horizontally in the basal bone in the mandibular anterior teeth.
-Each patient was exposed to densitometry measurement by DXA (Dual Energy X-ray Absortiometery) to estimate bone density in the spine and proximal femur.
Results:
- A positive correlation was shown between osteoporosis risk factors and BMD for the mandible after menopause.
- A positive correlation was demonstrated between mandibular bone trabecular architecture; width, spacing, geometry, and orientation.
- There was an indication for the relationship between skeletal bone mass and mandibular bone mass.
Conclusions:
- Mandibular BMD might help in detecting people at risk to fracture.
- The correlation between BMD and trabecular architecture and skeletal BMD might aid in determining the prognosis of dental treatment especially for implant restorations.

PP-197
FABRICATION OF MULTI-LAYERED AND NEOVASCULARIZED MYOCARDIAL TISSUES BY CELL SHEET ENGINEERING

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Recent progress in stem cell biology is likely to provide implantable sources of human cardiomyocytes in the near future. This possibility has encouraged current research on myocardial tissue engineering. Direct transplantation of dissociated cells is one approach and myoblast has been clinically used as alternative cell source.
On the other hand, further attempts to bioengineer 3-dimensional (3-D) heart tissues from cultured cardiomyocytes have also now begun. Myocardial tissue engineering based on the technology using 3-D biodegradable scaffolds as a temporary extracellular matrix (ECM) has been reported. In contrast to this context, we have exploited a novel cell manipulation technique to construct 3-D tissues from culture by layering cell sheets without any biodegradable alternatives for ECM. We apply novel cell culture surfaces grafted with temperature-responsive polymer, poly(*N*-isopropylacrylamide)(PIPAAm), from which confluent cells detach as a cell sheet simply by reducing temperature without any enzymatic treatments. We reported that layered neonatal rat cardiomyocyte sheets communicated electrically and engineered myocardial constructs pulsated simultaneously in macroscopic view. Long survival and macroscopic pulsation of 4-layer cardiac grafts in the subcutaneous tissues of nude rats was confirmed at least up to 6 months.
Although homogeneous, intimately cell-integrated 3-D myocardial tissues have been conveniently achieved, the thickness of the layered viable construct will be limited by oxygen permeation. As discussed elsewhere, the survival rate of cardiomyocytes located more than 50 ì m from a source of gas exchange decreases. To solve this crucial problem, we attempted to fabricate thicker myocardial constructs in vivo by repeating layered-tissue implantations at intervals enough for neovascularization. One day after a 3-layer myocardial construct was transplanted into the subcutaneous tissue of a nude rat, another 3-layer construct was overlaid. When the transplantation site was opened one week after the second operation, the whole construct pulsated spontaneously and simultaneously. Graft beating rate was 120 ± 10 bpm and fractional shortening was 6.1 ± 1.0%. The electrical stimulation to the one graft was transmit-